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THE FOURTH INTERNATIONAL CONGRESS OF ANATOMISTS

The Fourth Federative International Congress of Anatomists was held at Milan, Italy, September 3 to 8, 1936, under the presidency of Professor Ferdinando Livini. About 350 members attended. The participating national associations of anatomy included the American, British, Dutch, French, German, Italian, Japanese, Polish, Portuguese and Spanish. The official languages were English, French, German and Italian.

The papers were presented in two sections, with forenoon and afternoon sessions in the Anatomical Institute and the Institute of Forensic Medicine. Demonstrations were also given on three afternoons. The technical facilities were excellent.

The program of the local committee was marked by lavish hospitality. There were two evening receptions and a concert, in addition to the official banquet. The members of the Congress were also guests on numerous excursions. These included a half-day trip to the Certosa of Pavia and the University of Pavia, and a whole day in the Lake region (Como, Brunate, Lecco, Bergamo and San Pellegrino). Each member was also given a commemorative medal bearing the likeness of Malpighi, a free pass on the street car and bus system of Milan, and a sumptuous folio, with a wealth of illustration, descriptive of Vatican City. A special program of entertainment was added for the ladies in attendance at the Congress.

The official business was transacted by the group of 'Presidential Committees,' composed of the representatives appointed by the participating national associations. The

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members of the presidential committees were assigned as presiding officers for the various sectional meetings. It was decided to hold the next International Congress in 1940. Invitations from London and Japan for the next Congress were presented, but the final decision was referred to the constituent national societies.

Since the number of papers presented was large, it sometimes became necessary to reduce the scheduled time of 15 minutes for each, with 5 minutes for discussion. To correct this difficulty it was proposed that in future congresses, either the number of sections be increased, or a selection be made by requiring each society to choose from its own papers those considered the most appropriate for presentation.

An important event was the establishment of an International Commission on Anatomical Nomenclature. The American proposal for such a commission, to consist of one representative for each of the participating societies of anatomy, was finally accepted. The following members were appointed: Boeke (Holland), Fontes (Portugal and Spain), Hirako (Japan), Jackson (U.S.A.), Johnston (Great Britain), Loth (Poland), Pensa (Italy), Rouvière (France), and Stieve (Germany). Professor Stieve was made Chairman of the Commission.

At a subsequent meeting of the Commison (on September 4th) there was extensive discussion of the principles and problems involved. The following points were finally agreed upon.

1. The International Commission on Anatomical Nomenclature shall be a permanent organization, the membership being subject to change at each International Congress.

2. In the initial formulation of an international anatomical nomenclature, the Nomina Anatomica of the Anatomische Gesellschaft shall be taken as the basis for revision.

3. Any proposed changes shall be forwarded (through the corresponding national representative) to Chairman Stieve before September 1, 1937, for consideration by the Commission.

The question as to the publication of the proceedings of the International Congress was discussed at length by the Presidential Committees. It was generally agreed that a separate all-inclusive volume is most desirable to preserve the international character of the Congress. But this was regarded as impracticable for the present session. It was therefore decided that, as heretofore, each national society should publish its report independently, including the presentation of papers by its own membership. Those by the American members were as follows (including titles, abstracts and discussion).

PAPERS PRESENTED

1. ADDISON, W. H. F. (Philadelphia). *Enlarged hypophyses in old albino rats.* Abstract: Extraordinarily large hypophyses occur infrequently in both sexes of old rats in The Wistar Institute colony. The weight of the hypophyses averages twenty times that found in other animals of the same age. The rats were of normal body size. Microscopic sections show, in the pars anterior propria, the presence of numerous thin-walled blood channels, many widely distended with blood, as well as a great increase in gland-cell population, nearly all of the chromophobe undifferentiated type.

Discussion: Professor Defrise (Milano) chiede se furono studiati indici di correlazione fra gli aumenti di volume di ghiandole ipofisi e modificazioni volumetriche in altre ghiandole endocrine, in vecchi animali.

Addison. It will be very interesting to see the structure of the other endocrine glands, but these studies have not yet been made.

2. CUMMINS, H. (New Orleans). *Fingerprints correlated with handedness.* Abstract: Some 450 persons—tested for handedness, relative arm length, footedness and eyedness—are grouped according to functional asymmetry. The finger prints in these groups are compared with particular attention to the frequencies of whorls. Whorls are notably increased in left-handed females, with an accentuation of bimanual inequality in their occurrence. It is evident that functional asymmetry is foreshadowed early in the fetal period, since the dermatoglyphics are then definitely formed.

3. JACKSON, C. M. (Minneapolis). *Recovery of body weight in rats upon refeeding after prolonged suppression of growth by dietary deficiency.* Abstract: At weaning (age 3 weeks) albino rats were held at constant weight 15 weeks by protein-deficient diet, then refed on normal stock diet. Littermate controls were on stock diet throughout. Mortality was high in the test rats, especially the males. On refeeding, the surviving test rats grew rapidly, and the females overtook the normal controls when about 9 months old. The males, however, appeared permanently stunted, remaining below the controls at 1 year. Simple underfeeding (instead of protein deficiency) gave somewhat similar results.

Discussion: Professor Macklin (London, Ontario) asked what cell damage is responsible for the irrecoverable stunting of growth after protein starvation in the young male as contrasted with the female. Does this difference rest on permanent injury to any specific group of cells, such as those of the thyroid or hypophysis, or other organ or tissue?

Professor Stieve (Berlin). Die Untersuchungen über die der Herr Vortragende berichtete, bestätigen in sehr schöner Weise die Beobachtungen, die bei Kindern gemacht wurden. Verschiedene Kinderärzte haben schon darauf hingewiesen, dass Kinder, die während längerer Zeit schlecht ernährt wurden, besonders rasch wachsen und das Versäumte noch nachholen, wenn sie vor Abschluss des Wachstums kräftig ernährt werden. Stefko hat dieselben Beobachtungen an russischen Kindern gemacht, die längere Zeit gehungert hatten. Während der Zeit des Hungers nehmen die Kinder zwar nicht an Gewicht zu; ihre Extremitäten wachsen aber besonders stark, sie werden länger als die normal ernährten Kindern. Ich möchte den Herrn Vortragenden fragen, ob auch er solche Beobachtungen machen konnte.

In reply to Professor Macklin, I have not yet made microscopic studies of the different organs, and am therefore unable to say what injuries are responsible for the permanent dwarfing in the males.

In reply to Professor Stieve, these experimental studies in general show that the skeleton persists in growth, even when the body weight is held constant. Nevertheless upon refeeding the bones fail to recover their normal adult size (weight), which would indicate some irreparable injury resulting from the period of inanition.

4. Lewis, F. T. (Boston). *The shape of cells.* Abstract: Dodecahedral forms of compressed plastic solids contrast with the tetrakaidecahedral shapes of bubbles in foam. The average cell is 14-hedral, with facets conforming to Euler's theorem. When tension makes all intersections 3-rayed, the polygons covering a cell have twelve less sides than if they were all hexagons. Tension along edges produces *edge canals* (intercellular spaces) differing from interfacial intercellular spaces associated with bridges. Irregular vacuoles disguise the 14-hedral shape which however is probably the basic form for cells in masses.

5. Macklin, C. C. (London, Ontario). *a. The mammalian pulmonic alveolar wall.* Abstract: The wall of the pulmonic alveolus in the domestic cat, rabbit and man is composed of syncytially joined flattened septal cells, reinforced by elastic and reticular fibrils, and tunneled by capillaries, whose walls are condensations of the protoplasm. Many change into sessile, or even free, phagocytes. Epithelium was not seen. Pores are perforations of the septal cell protoplasm of the capillary meshes. Osmic acid vapor insufflated directly into the lung of the living animal gave good fixation.

b. The perivascular interstitial tissue of the lungs as an air passage in the experimental production of massive collapse with pneumothorax. Abstract: During continuous insufflation of air, pure or containing osmic acid, into the right lower lobe of the cat's lung, both lungs became collapsed and both pleural cavities dilated with air. Interstitial emphysema occurred in the mediastinum, neck, axillae and retroperitoneum. The perivascular tissue (particularly the periarterial) of the insufflated region was distended with air, apparently from the alveoli, and this had probably worked its way to the parts mentioned, and to the perivascular tissue of some of the remaining lobes.

(Members of the Congress are entitled to present abstracts of two papers, but may read only one: Doctor Macklin chose to speak on 'The perivascular tissue.')

6. Streeter, G. L. (Baltimore). *Early stages of the macaque embryo.* Abstract: A review of the embryology of Macacus rhesus, beginning with the free blastocyst of the ninth day and extending to the primary tissue differentiation and embryogenesis up to the formation of the early somites. It is found that the segregation of the auxiliary parts of the ovum from those cells which are to form the embryo antecedes, and so is relatively independent of the subsequent grouping of the body tissues into three germ layers.

Discussion: Professor Florian (Bratislava). Does Professor Streeter believe in a double origin of endoderm in the macaque, one part originating from ectoderm and the other part originating from a layer which seems to give origin also to mesoderm?

Professor Stieve (Berlin). Die besonders schönen Präparate des Herrn Vortragenden erklären uns in besonders schöner Weise viele Vorgänge von denen wir bisher nur einzelne Stadien kannten, und die uns aus den gezeigten Bildern erst verständlich werden. Ich darf den Herrn Vortragenden nur fragen ob sich die Amnionhöhle bei den untersuchten Arten als reiner Spaltamnion anlegt und des weiteren möchte ich über der Entwicklung des Dottersackes fragen. Der Keim besteht zuerst ja aus zwei Spaltblättern, dem Ectoderm und dem Entoderm. In dem Ectoderm entwickelt sich die Amnionhöhle. Dann lösen sich, soweit ich die Bilder verstanden habe, aus dem Entoderm Zellen ab, diese bilden den Dottersack. Somit entsteht also der Dottersack, in der Tat wie ich dies angenommen habe, indem dass vom Rande des Entodermes Zellen auswachsen.

Professor von Möllendorff (Zürich) betont Unterschiede in Implantationsweise gegenüber dem menschlichen Ei.

Professor Gérard (Brussels). La belle démonstration du Professeur Streeter permet de considerer que, dans un oeuf de Mammifère en voie de développement, il faut distinguer deux regions bien distincte: l'une est formée par l'embryon proprement dit, qui se differencie suivant une loi fixe, la même pour toutes les espèces; l'autre constitue la partie extraembryonnaire—partie auxiliaire de Streeter—qui donnera l'ensemble des annexes foetales au sens étendu du mot. Cette dernière se differencie suivant les modes les plus variés, dans les differents espèces. Cette manière de voir permet, je pense, de concilier les points de vue si divers exprimés par les differents auteurs dans leurs études sur le développement des annexes foetales de Mammifère.

Professor Ranzi (Perugia) confermando quanto ha detto il Professor Gérard mette in evidenza che fuori dei Mammiferi si osservano fatti stupendi di embrioni che presentano modilità di sviluppo identiche e profonde diversità di comportamento degli annessi embrionali. Basta ricordare i due Mustelus: M. laevis con placenta, M. vulgaris senza placenta.

Professor Streeter. In reply to Professor Stieve, the amniotic cavity is regarded as a cleft appearing between the definitive embryonic epithelium and the overlying trophoblast. Its cells are derived entirely from trophoblast. It is probable that a similar process takes place in man, the later stages in the two being closely similar. The chief difference between the two is the double placentation of the macaque. Instead of penetrating beneath the epithelial surface completely as it does in man, the ovum of the macaque implants on both of the two opposing surfaces of the uterine wall. The embryonal one-half attaches first and forms the primary placenta, the abembryonal one-half attaches soon after to the other wall and forms the secondary placenta. The only other difference between man and macaque appears to be the phenomenon of epithelial proliferation which characterizes the macaque before the appearance of villi and which does not happen in man.

In reply to Professor Florian, the endoderm of the yolk sac is regarded as having two points of origin: a) The gut-endoderm probably coming from the definitive ectoderm; and b) the yolk sac endoderm which is derived from the auxiliary substances of the egg, and this is more closely related to trophoblast than to embryonic ectoderm.

7. VICARI, E. M. (New York). *Parafollicular thyroid cells in pregnant dogs.* Abstract: Structurally different breeds of dogs show characteristic histological variations in their thyroids. These variations are apparent both in follicular and parafollicular cells. In glands showing activation of the follicular epithelium, parafollicular cells are usually numerous. The occurrence of pregnancy tends to further increase the number of parafollicular cells. These observations indicate a relationship between the abundance of parafollicular cells and modifications in the physiologic condition of the individual, as in pregnancy.

Discussion: Professor Boeke (Amsterdam) asked how many individuals Doctor Vicari has studied, because there is so much variation in the thyroid gland in different individuals.

Professor Policard (Lyon) demande le sentiment de Docteur Vicari sur les rapports histologiques entre cellules parafolliculaires et follicules thyroidiens, et si elle accepte les conclusions du travail de M. Thomas, de Gand, sur ces rapports.

Doctor Vicari. In reply to Professor Boeke and Professor Policard, about 400 individual dogs of different structure were studied with regard to variation in abundance of the parafollicular thyroid cells. About 40 pregnant individuals were studied. These are observations only and await generalization and confirmation from experimental work.

8. WINDLE, W. F. (Chicago). *The genesis of somatic movements in sheep embryos.* Abstract: Embryonic skeletal muscle contractions were elicited by faradic stimulation before the central nervous system functioned reflexly. Active reflex-like movements appeared in 23.5- to 25-mm. embryos, and were localized responses to mechanical and electrical stimulation. Integration of these embryonic movements followed their appearance as separate phenomena. The reactions in question are thought to be reflexes of spinal type. In other mammals (rat and cat) their development is coincidental with the structural development of the first spinal reflex arcs.

The American Committee
C. M. JACKSON, *Chairman*
F. T. LEWIS
G. L. STREETER

THE TOPOGRAPHICAL POSITIONS AND THE MUTUAL RELATIONS OF THE VISCERAL BRANCHES OF THE ABDOMINAL AORTA. A STUDY OF 100 CONSECUTIVE CADAVERS [1]

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ONE PLATE (EIGHT FIGURES)

The regular textbooks of gross anatomy offer little precise information concerning the points at which the visceral branches of the abdominal aorta arise, either in relation to the vertebrae or the bifurcation, or in relation to one another.[2]

The present article aims to furnish such information. It is based upon a study of 100 consecutive specimens from which were collected data upon the position of the abdominal arotic branches, and of the aortic bifurcation in relation to the upper, middle, and lower thirds of vertebrae (table 1),[3] upon the distance above the bifurcation at which each branch arose (table 2), and upon the distance between adjacent visceral branches (table 3).

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[1] Contribution no. 237 from the anatomical laboratory of Northwestern University Medical School.

[2] The points of aortic origin are commonly described as follows: That of the coeliac as being 'just below the aortic hiatus,' that of the inferior mesenteric '37 cm. above the bifurcation;' the superior mesenteric is then described as arising 'a little below the coeliac,' the renals 'just below the superior mesenteric,' the internal spermatics (and ovarians) ''a little below the renal arteries''—all of which is little more exact than would be a serial enumeration of these vessels in the order of their origin from above downward. Only one text bases its descriptions consistently on the relation of the vessels to dependable landmarks, the segments of the lumbar part of the vertebral column, but at the same time fails to discuss the wide range of variation which exists in these relations (see footnote 4).

Relation to vertebrae (table 1). The coeliac artery in 74% of the cases arose opposite the first lumbar vertebra; the superior mesenteric in 83% originated opposite the vertebral area from the middle of the first to the upper third of the second lumbar vertebra, and the inferior mesenteric in 73% opposite the vertebral area from the middle of the third lumbar vertebra to the intervertebral fibrocartilage between the third and fourth lumbar vertebrae; the internal spermatic (and ovarian) vessels in 64% of the cases took origin opposite the area from the lower third of the second lumbar to the upper third of the third lumbar vertebra. The bifurcation in 84% of the cases occurred in the area between the middle third of the fourth lumbar and the upper third of the fifth lumbar vertebra. Of the four sets of aortic branches just described, the coeliac and inferior mesenteric arteries were most concentrated in their areas of origin; then followed in order the superior mesenteric and the internal spermatic (and ovarian) arteries. Most variable in their positions of all the visceral branches, were the renal arteries (i.e., as shown in table 1, their area of possible origin was greatest); although 64% of the renal arteries lay opposite the vertebral column between the intervertebral fibrocartilage of the first and second lumbar vertebra and the middle third of the latter vertebra, the most superiorly placed examples of these arteries arose opposite the middle of the first lumbar, the

---

[3] Our records were secured from the cadavers dissected in the anatomical laboratory by the first year students in medicine. An accurate pencil drawing was prepared of each aorta and the stumps of the branches; the distance from the bifurcation of the aorta to the lower border of the body of the fifth lumbar vertebra was recorded. Using the latter measurement again as reference, after the cadavers had been hemisected, the outlines of the vertebrae were added to the drawings; therefrom the positions of the visceral branches of the aorta were recorded in relation to thirds of the vertebrae. Data on the mutual relations of the branches and their relation to the bifurcation were taken from the same records.

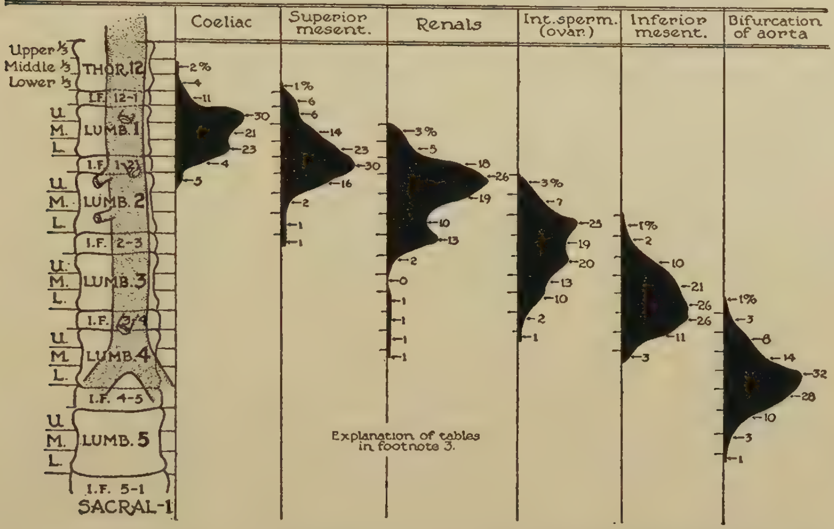
Data were converted from simple tabulations into curves (tables 1, 2 and 3), in which the abscissas were placed vertically, the ordinates horizontally, in order that the frequencies would be more graphically shown through the relationship of the height of the curve to the vertebrae (table 1), to the bifurcation (table 2), and to the distances between adjacent aortic branches (table 3). In tables 1 and 2 the commonest position of each aortic visceral branch, as shown by the curve, was included with the drawing of the aorta.

All curves were based upon the number of vessels studied, but percentages, not numbers of vessels, were recorded in the tables in association with the curves. In table 1, the curves for the coeliac and the mesenteric arteries and the aortic bifurcation were based upon 100 examples from as many bodies; the curve representing the renal arteries, upon 150 vessels from sixty-eight bodies—there being more than twice as many renal arteries as there were cadavers, because accessory vessels were included in the count (see footnote 4); the curve for the spermatic arteries was based upon ninety-four vessels from sixty-eight bodies—there being less than twice as many vessels as cadavers, since frequently only one artery of a pair was preserved by the dissectors. In tables 2 and 3 the curves for the coeliac and the mesenteric arteries were based upon seventy examples; in the former table, the curve for the renal arteries was based upon 162 vessels, that for the spermatic arteries upon eighty-four vessels.

In table 1, I.F. stands for intervertebral fibrocartilage.

TABLE 1

Visceral branches and bifurcation of aorta in relation to vertebrae



Explanation of tables in footnote 3.

most inferior ones opposite the middle of the fourth lumbar vertebra.[4]

As would be expected, there is no regular association between the length of the aorta and the relation of the visceral branches to the vertebrae. Thus, although in specimens I and II the coeliac artery was removed from the aortic bifurcation by approximately the same distance (13.7 cm.), the vertebral relations differed by almost a whole vertebral segment (compare figs. 2 and 4). In specimen IV, an aorta but two-thirds as long as that of specimen I, the relations of the coeliac (and other major vessels) and of the bifurcation to the vertebral segments were substantially the same as those obtaining in the case of the longer vessel (compare figs. 2 and 8). In specimen III, an aorta the same length as specimen IV, the coeliac artery and aortic bifurcation were each situated more cranially by the distance of one vertebral segment (compare figs. 6 and 8).

Relation to aortic bifurcation (table 2). Of less value than the preceding are data upon the relations of the visceral branches to the aortic bifurcation,[5] since the latter is itself a highly variable landmark.

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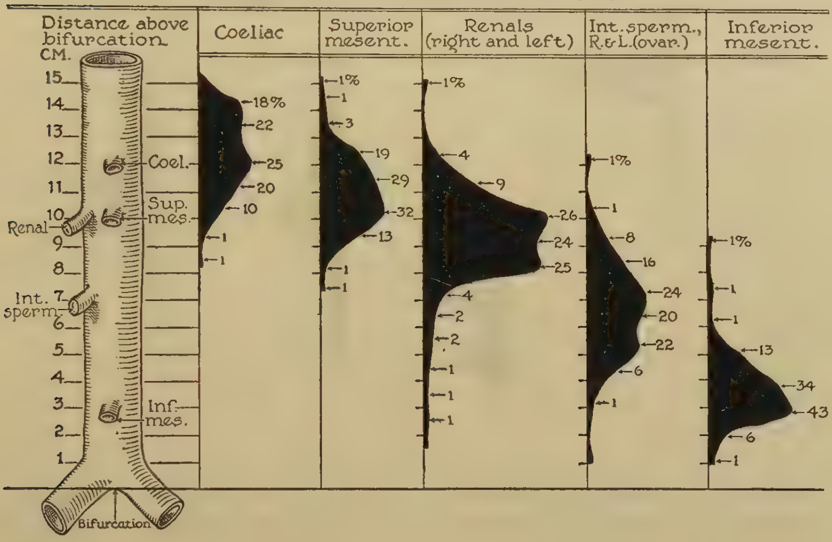
[4] From a study of 200 consecutive cadavers (see p. 11) it was found that in 65% a unilateral or a bilateral 'anomaly' of the renal blood supply occurred, in the form of one or more supernumerary renal arteries; of the 400 kidneys, considered without regard to side, 47% were supplied with extra renal branches (note renal vessels in figs. 1 and 3). It was also found that considerable variation occurs in the distance between the aortic extremities of the supernumerary renal branches—from 0.7 cm. to 8.9 cm. in normally placed kidneys and an even greater distance in cases of pelvic and horseshoe kidney. The only one of our common textbooks which presents, consistently, the relation of the several visceral vessels to vertebral levels, states that the renals arise at the first lumbar vertebra or somewhat lower—certainly an almost arbitrary choice. The same authority presents the following relations for the other vessels: The coeliac, between the twelfth thoracic and first lumbar vertebrae; the superior mesenteric between the third and fourth lumbar; the internal spermatics at the second lumbar vertebra.

[5] Obviously less dependable since the reference point (the aortic bifurcation) is itself variable (table 1); the vessels, which are themselves highly variable, must then be described in reference to the forking of an axial vessel which may occur anywhere between the lower third of the third lumbar and the lower third of the fifth lumbar vertebra.

Of the several sets of distances intervening between the bifurcation and the visceral branches, the interval separating the bifurcation from the coeliac artery exhibited least variation; in 85% the cases varied between 11.5 and 14.5 cm. Next in order of increasing variability were the distances separating the superior mesenteric arteries from the bifurcation; in 80% of the cases the distance varied between 10.5 and 12.5 cm. Third in succession was the inferior mesenteric artery; although 90% of the examples were separated from the bifurcation by a distance of 2.5 to 5.5 cm., the remaining 10% were spread over a considerable fraction of the aorta's length. Last but one in the succession based on increasing variability was the internal spermatic artery; last in the series, exhibiting greatest variation, was the renal artery (see footnote 4).

TABLE 2

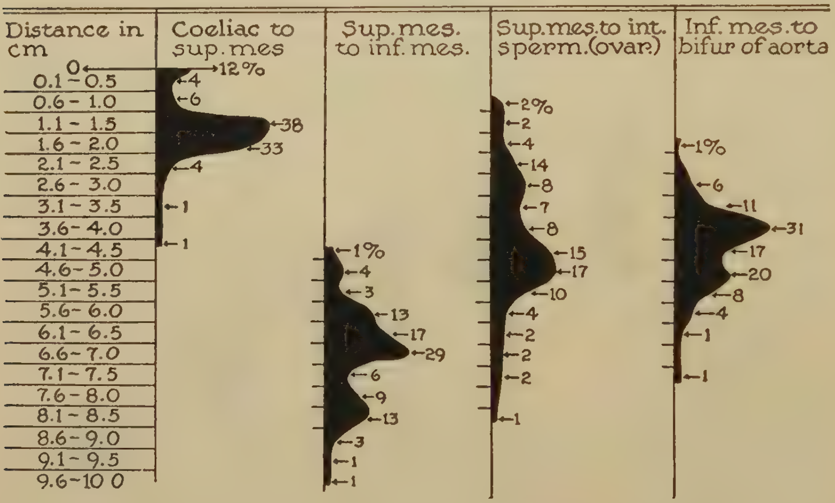
Distance above bifurcation at which visceral branches of abdominal aorta arise.



The average distances from the bifurcation to the origins of the several arteries were found to be as follows: Coeliac 12.7 cm.; superior mesenteric, 11.3 cm.; inferior mesenteric, 4.3 cm.; renals, 9.5 cm.; internal spermatics (and ovarians), 7.1 cm. The point (in relation to the bifurcation) at which the greatest number of examples of each branch arose is shown by the diagrammatic figure of the aorta in table 2; these points are: Coeliac, 12.5 cm. above the division; superior mesenteric and renals, 10.5 cm.; internal spermatics (ovarians), 7.5 cm.; inferior mesenteric, 3.5 cm.

TABLE 3

Distances between visceral branches of abdominal aorta



Mutual relations (table 3). Of a kind with the preceding (since each vessel is variable in its place of origin) are those data which record the distances between adjacent aortic branches.

Least variable was the distance between the coeliac and superior mesenteric arteries, being, in the greatest number of instances (71%) from 1.0 to 2.0 cm.;[6] in 12% of the cases the two vessels arose from a common stem. Somewhat more variable were the distances between the superior and the inferior mesenteric arteries, and between the latter and the bifurcation of the aorta. Since the internal spermatic arteries vary greatly in their distance from the bifurcation (table 2) the recorded variation in the distance from the superior mesenteric to the internal spermatic is of course expected (table 3).[7]

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[6] The distance between the coeliac and the superior mesenteric arteries is usually stated to be 1.25 to 1.5 cm.—no other comparable measurements being presented.

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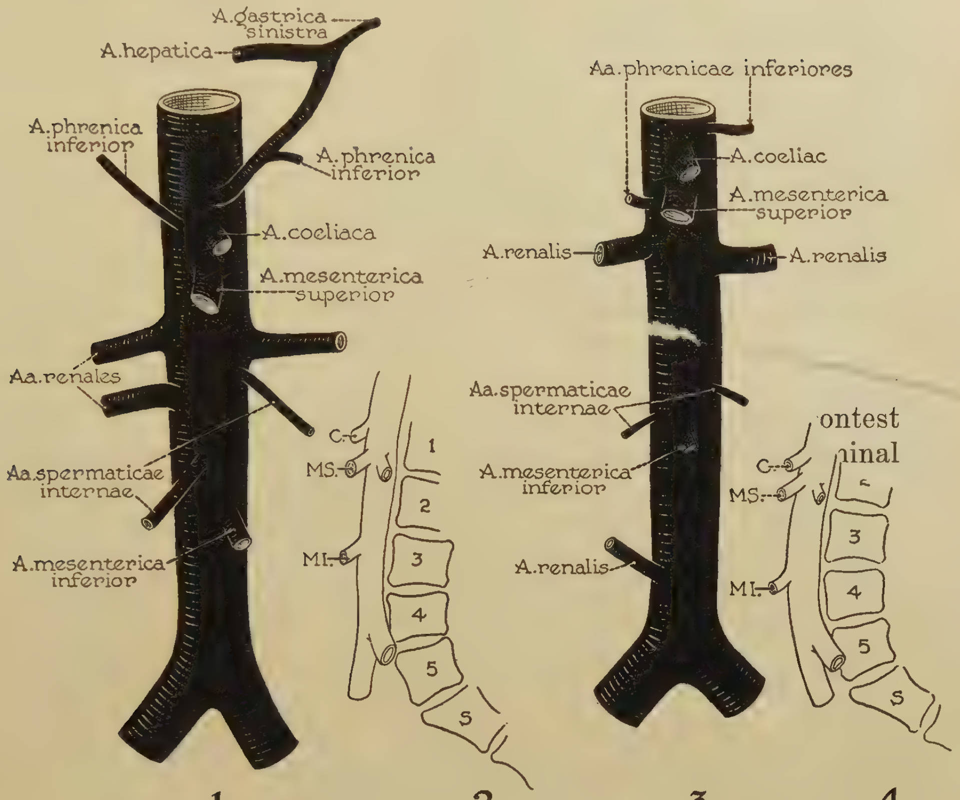
[7] No regular interdistances can be offered for the renal arteries, since they may arise almost in conjunction with the coeliac, from the iliac arteries, or from any portion of the aorta intervening between these extremes (see footnote 1).

PLATE 1

EXPLANATION OF FIGURES

1 to 8 Abdominal aortae, four examples. Specimens I (figs. 1 and 2) and II (figs. 3 and 4), the longest aortae encountered, from males of medium stature; specimens III (figs. 5 and 6) and IV (figs. 7 and 8), the shortest examples, from females of low stature. Abbreviations: C, a. coeliaca; MS, a. mesenterica superior; MI, a. mesenterica inferior. Figures 1, 3, 5 and 7, anterior views, one-half natural size; figures 2, 4, 6 and 8, lateral views of hemisections, one-quarter natural size.


A.gastrica sinistra
A.hepatica
A.phrenica inferior
A.phrenica inferior
A.coeliaca
A.mesenterica superior
Aa.renales
Aa.spermaticae internae
A.mesenterica inferior
C.
MS.
MI.
1
2
3
4
5
S
Aa.phrenicae inferiores
A.coeliac
A.mesenterica superior
A.renalis
A.renalis
Aa.spermaticae internae
A.mesenterica inferior
A.renalis
C.
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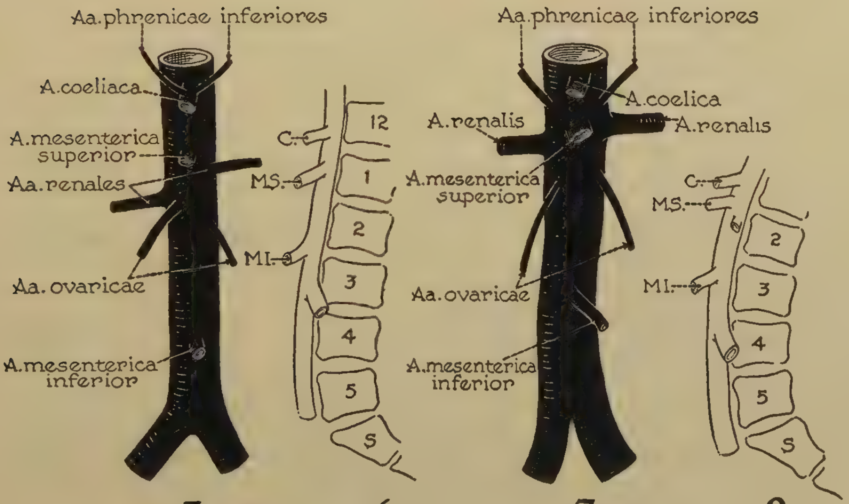
1 2 3 4


Aa.phrenicae inferiores
A.coeliaca
A.mesenterica superior
Aa.renales
Aa. ovaricae
A.mesenterica inferior
C.
MS.
MI.
12
1
2
3
4
5
S
Aa.phrenicae inferiores
A.coelica
A.renalis
A.renalis
A.mesenterica superior
Aa.ovaricae
A.mesenterica inferior
C.
MS.
MI.
2
3
4
5
S

5 6 7 8

THE ABDOMINAL VISCERA IN SITU. A STUDY OF 125 CONSECUTIVE CADAVERS[1]

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ONE PLATE

Perhaps no teacher of anatomy or of surgery would contest the statement that the preliminary study of the abdominal organs in the supine body is a subject of major importance to the medical student—since the anatomical position is also the surgical one in laparotomy. Yet the student, by the usual sources of anatomic information, is neither greatly aided in his problem of understanding the positions of the organs viewed in situ, nor forewarned of the variations in position that regularly present themselves to the dissector; he is led to expect invariably a projecting liver, a bulbous stomach, an apron-like omentum, and is baffled by the frequent occurrence of departures from this plan.[2]

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[1] Contribution no. 238 from the anatomical laboratory, Northwestern University Medical School.

[2] For 10 years we have taught the anatomy of the living to the first year students in medicine, but distinctly as an auxiliary of, and correlated with the anatomy of the cadaver. It early became evident that, although abundant statistical information was available on variations in the form and the position of the organs of the living person standing erect, sparse indeed was comparable information secured from human bodies, in the anatomical laboratory placed supine. Furthermore it was clear that the student did not realize that the radiographic stomach—an elongate bag, dragged, by its barium meal, toward or into the pelvis of the erect patient—would become, when the same patient lay face upward, the stomach familiar to the dissector, that is, a much shorter, plumper organ situated in the left hypochondrial region; and that all of the upper abdominal viscera likewise move, with the change in posture, from a position of seeming ptosis into one in which they gravitate to the space just beneath the diaphragm. In other words, the student is wont to consider the anatomic shapes and relations observed in the roentgenographic room essentially different from those displayed in the dissection laboratory. Enamored of a captivating apparatus the novice is too likely to favor the information obtained by radiography to the point of disregarding that secured by the far older, less theatrical, method dissection.

From our dissection rooms we have gathered records on the positions of the abdominal organs; those which deal with the more superficial structures we now wish to present; while recognizing that the collection is not yet extensive, it may nevertheless be of service until such time as more extensive series are available.[3] They fall readily into groups, types of which are shown in our illustrations.

TABLE 1

Superficial abdominal contents; distances inferior to xiphisternal articulation. One hundred and twenty-five cadavers[1]

LIVER				STOMACH		OMENTUM		TRANSVERSE COLON	
Right lobe		Left lobe							
Distance in centimeters	Per cent of cases	Distance	Per cent	Distance	Per cent	Distance	Per cent	Distance	Per cent
0	38	0	5	0	16	0	17	0	12
0.5– 2.5	20	0.5– 2.5	4	5	2	3 – 5	1	5.5– 7.5	4
3 – 5	26	3 – 5	4	5.5– 7.5	5	5.5– 7.5	0	8 –10	5
5.5– 7.5	11	5.5– 7.5	9	8 –10	10	8 –10	0	10.5–12.5	18
8 –10	4	8 –10	20	10.5–12.5	15	10.5–12.5	1	13 –15	16
10.5–11	1	10.5–12.5	30	13 –15	20	13 –15	2	15.5–17.5	11
		13 –15	20	15.5–17.5	20	15.5–17.5	2	18 –20	15
		15.5–17.5	7	18 –20	6	18 –20	6	20.5–22.5	20
		18 –18.5	1	20.5–22.5	5	20.5–22.5	6	23 –25	10
				23 –25	1	23 –25	13	25.5–27.5	8
						25.5–27.5	10	28 –30	5
						28 –30	7	30.5–32.5	0
						30.5–32.5	14	33	1
						33 –35	17		
						35.5–36	4		

[1] In the case of the liver zero indicates that the lobe did not extend below the costal margin, in the cases of the stomach and transverse colon that it was concealed by the liver; in the case of the omentum, zero usually indicates that it was tucked away, out of view, between the liver and the transverse colon. All measurements were made from xiphisternal articulation to the lower border of the organ or subdivision of the organ. For convenience in tabulation the smallest unit used in measurement was a half centimeter.

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[3] The records of 125 consecutive normal sets of abdominal organs upon which this account is based were collected by the simple method of tracing with wax pencil upon glass the outlines of the exposed viscera and omentum in situ and then transferring these to translucent paper; the examples used in the illustrations were reduced to one-half natural size by precision pantograph, then to one-sixth natural size in reproduction.

Liver. In 94% of our present series of 125 sets of organs the left lobe of the liver projected below the xiphisternal articulation (infrasternal notch) to an extreme descent of 18.5 cm (fig. E; also table 1). In only 6% of the cases was it entirely concealed in anterior view, reaching an extreme height of 2 cm. superior to the notch (fig. A).[4]

Stomach. In 16% of the cases the stomach was entirely concealed by the liver and the transverse colon (fig. F). Invariably covered in at least a portion of its anterior aspect by the liver—upon whose position its own seemed largely to depend—its greater curvature in 42% of the cases extended below the xiphisternal articulation for a distance of 5 cm. (fig. G) to 13.5 cm. (figs. H and I), and for a distance of 14 cm. to 25 cm. (fig. J) in 42%. The stomach variously occupied the infrasternal notch (fig. G), the subcostal space near the left lobe (fig. H), the notch between the lobes (fig. I), and a wider area inferior to the anterior margin (fig. J).

Transverse colon. In 13% of the cases (not illustrated) the transverse colon was not in view inferior to the liver or stomach; in 58% its lower margin extended for a distance of 8 cm. (fig. K) to 21 cm. (fig. L) below the xiphisternal articulation, in 29% for 21.5 cm. to 33 cm. (fig. M).[5]

Greater omentum. The greater omentum was not in view in 19% of the cases (fig. N), and appeared as a mere fringe in an additional 2% (fig. O); in 8% it extended 14 cm. to 19.5 cm. (fig. P) below the xiphisternal articulation; in the remaining 71% from 20 cm. to 36 cm. (figs. Q and R).

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[4] The illustrations of the liver (figs. A to E) were selected to show the costal relations of the left lobe, since the right lobe was concealed in 38% of the cases.

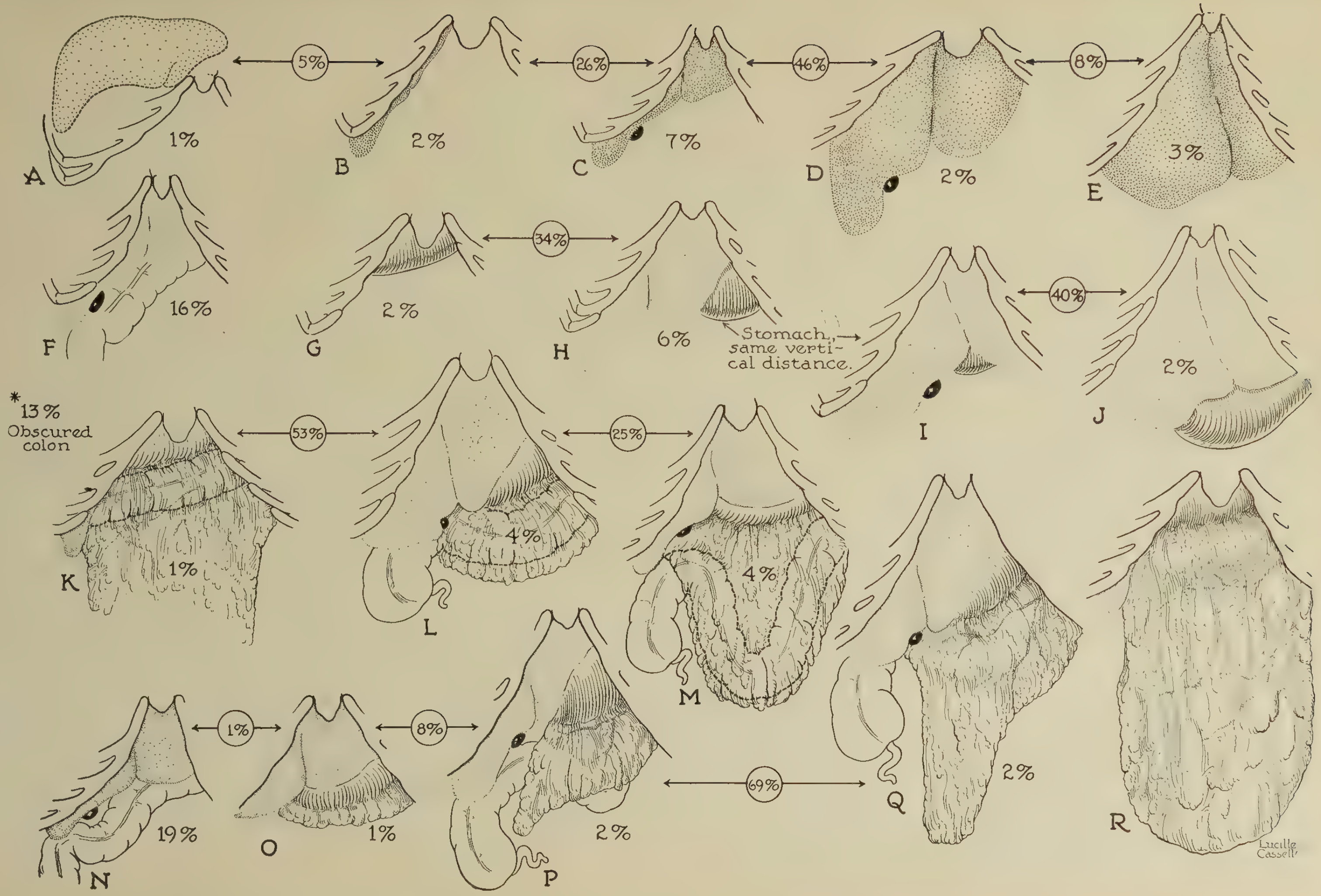
[5] In thirty-four of 120 specimens the caecum was not in view, being covered by the coils of small intestine or by the omentum; in the eighty-six cases in which it was showing, its inferior margin was situated superior to the anterior superior iliac spine in forty-two, opposite the spine in twenty-six, inferior to it in eighteen.

PLATE 1

EXPLANATION OF FIGURES

A to R Drawings of liver (figs. A to E), stomach (figs. F to J), transverse colon (figs. K to M), and omentum (figs. N to R). Within each set, the examples are arranged in order of increasing distance from xiphisternal articulation. One-sixth natural size.

A percentage placed near or upon a figure indicates the fraction of 125 cases which the illustration (of liver, etc.) represents; a percentage between two figures, encircled, indicates the fraction of the cases which fall between the adjacent figures. * The examples of obscured colon are not represented in the illustrations.


A
1%
5%
B
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C
7%
46%
D
2%
8%
E
3%
F
16%
G
2%
34%
H
6%
Stomach, same vertical distance.
I
40%
J
2%
*13% Obscured colon
K
1%
53%
L
4%
25%
M
4%
N
19%
1%
O
1%
8%
P
2%
69%
Q
2%
R
Lucille Cassell

THE EFFECTS OF ANTERIOR PITUITARY EXTRACT AND CERTAIN ENVIRONMENTAL CONDITIONS ON THE GENITAL SYSTEM OF THE HORNED LIZARD (PHRYNOSOMA CORNUTUM, HARLAN)

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ONE PLATE (EIGHT FIGURES)

INTRODUCTION

There is some disagreement as to the effects of injections of mammalian gonadotropic substances into reptiles. Herlant ('33) has reported that administration of pituitary extracts and pregnancy urine in lizards caused a marked increase in the amount of interstitial tissue with very little stimulation of the germinal epithelium. Schaefer ('33) on the other hand, found that spermatogenesis was induced in the hypophysectomized garter snake by anterior pituitary extracts. Evans ('35) was able to produce ovulation in the female lizard, Anolis, with sheep pituitary extract, and in the male he obtained an hypertrophy of the epididymis as well as an increase in the amount of the germinal epithelium by the injection of this preparation and antuitrin-S. Similar results were reported by Turner ('35) in the male lizard, Eumeces, following administration of antuitrin-S during the period of sexual regression.

There have been many attempts to determine the environmental factors ncessary for the production of sexual cycles in certain monoestrous or annual breeding vertebrates but to the writer's knowledge no similar studies have been made on reptiles.

THE ANATOMICAL RECORD, VOL. 67, NO. 1 AND SUPPLEMENT NO. 1

The present experiment was undertaken in order to observe the effects of: 1) Low temperature; 2) high temperature, light and food; and 3) the combination of high temperature, light and food with injections of hog anterior pituitary extract on the genital system of the horned lizard during the period of normal sexual quiescence.[1]

PROCEDURE

Mature animals of both sexes were placed in a covered box of sand at a temperature of 5°C. when collected from the normal habitat in November, 1934.[2] On January 17th they were divided into two groups, one of which was left as described above (group I) while the other was put in a compartment in which the temperature was kept at 35°C. by a continuously lighted 16 candle power electric bulb which was 2 feet above the animals. The animals of group I burrowed in the sand and hibernated for the duration of the experiment, while those kept at the higher temperature became active and fed on meal worms, beetles and blow-fly larvae—there being an oversupply of food present at all times. The animals were watered individually on alternate days.

On February 20th the lizards which were being kept under the conditions of high temperature with food and continuous lighting were divided into an experimental (group III) and a control group (group II). The individuals in group III received intraperitoneal injections of 0.25 cc. of a hog anterior pituitary extract[3] on this date and on alternate days thereafter until a total of 3 cc. had been given. Twenty-four hours after the last injection the animals of all three groups were killed and the gonadal and body weights were taken. The testes, epididymides and oviducts were fixed in Bouin's solution, sectioned at 8 to 10 μ and stained with haematoxylin and eosin.

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[1] The writer wishes to thank Drs. F. L. Hisaw, H. L. Fevold and R. K. Meyer for their help and suggestions throughout the course of this experiment.

[2] All the specimens used in the experiment were obtained through the courtesy of Dr. G. E. Potter, Baylor University, Waco, Texas.

[3] The extract was prepared according to the method of Fevold, Hisaw and their co-workers ('33); the process of fractionation being discontinued after the first isoelectric precipitation, so as to afford a semi-purified, non-toxic extract of the whole anterior pituitary. One cubic centimeter was equal to 1 gm. equivalent of desiccated whole hog anterior pituitary gland or 20 rat units. One rat unit is that amount which when injected into a 22-day-old female rat will produce a 100% increase in the weight of the ovaries.

RESULTS

Males. The gross results are indicated in table 1. In group I a photomicrograph of a seminiferous tubule (fig. 1) shows the typically inactive state as described by Blount ('29) for Phrynosoma solare. In such a testis the tubules are small and completely filled with cells. The spermatogonia are with few exceptions in the resting condition and the remainder of the cells are in the leptotene or pachytene stage.

TABLE 1

Males

GROUP III			GROUP II			GROUP I		
Temperature 35°C. + food + light						Temp. 5°C. – hibernating		
Injected., A.P. extracted			Non-injected			Non-injected		
Number of animals	Body weight	Testis weight (2)	Number of animals	Body weight	Testis weight (2)	Number of animals	Body weight	Testis weight (2)
	gm.	*mg.*		*gm.*	*mg.*		*gm.*	*mg.*
37	25	586	23	14	23.9	6	23.5	60
38	20	332	25	16	30.7			
39	17	545	26	19	38.2	8	21.5	47
41	22	500	27	17	32.1	9	23.5	58
34	23	574	28	18	28.7	10	16	32
29	23	507	1	23	57.0	53	15	30
2	20	540						

In the testes of group II the seminiferous tubules, though still small, contain lumina and a few dividing cells. Spermatogenesis has not progressed beyond the pachytene stage (fig. 2).

On the other hand, in injected animals (group III), the gross diameter of the tubules is greater than in those of group II and the lumina show a relatively greater increase in size. There are many mitoses in the spermatogonia, the total number of cell generations is increased, and all different stages of spermatogenesis from gonia to mature sperm are represented (fig. 3). This condition agrees with Blount's description of the testis of the sexually active animal in June. Although no special effort was made to study the amount of the interstitial tissue, it was evident that the principal effect of the injections is on the germinal epithelium. The epididymides of groups I and II (non-injected) are composed of a non-secretory, cuboidal epithelium (fig. 5), whereas those of group III are of tall, columnar cells which are of the active secretory type (fig. 4). There is no histological difference between the epididymides of groups I and II.

TABLE 2

Females

GROUP III			GROUP II			GROUP I		
Temperature 35°C. + food + light						Temp. 5°C. − hibernating		
Injected., A.P. extracted			Non-injected			Non-injected		
Number of animals	Body weight	Ovary weight (2)	Number of animals	Body weight	Ovary weight (2)	Number of animals	Body weight	Ovary weight (2)
	gm.	*mg.*		*gm.*	*mg.*		*gm.*	*mg.*
5	16.5	1165	3	16	40	11	26.5	29
30	31	1031	4	27	50	12	35	93
31	23	289	20	27	121.6	13	19.5	21
32	34	425	21	22	19.9	49	26	38
33	23	1313	22	23	26	51	22	36
35	26	151	24	16	22			
36	38	1299						
40	21	139						
42	26	778						
43	25	632						
44	19	460						
45	25.5	720						

Females. The ovary of the lizard is similar to that of the bird in that there is little stroma; the bulk being made up of eggs in various stages of development. The increased weight of the ovaries of the injected group over that of the non-injected groups (table 2) is caused by an increase in the size of the eggs rather than an increase in their number. The ova of the latter are small—none being more than 1 mm. in diameter—whereas those of the injected animals are as large as 4 to 5 mm.

In the oviducts of groups I and II (fig. 6) there is a thick layer of connective tissue on the outside of the muscularis coat. The lumen is lined by a folded, apparently non-secretory, epithelium in which the cells are small and cuboidal.

The oviducts of group III have a very thin connective tissue layer, the epithelial lining is extremely convoluted, and the folds are pushed close together so as to form 'ducts' lined by apparently secretory cells (fig. 7). Two animals of this group evidenced a condition which differs somewhat from both of the types described above. In these (fig. 8) the connective tissue layer is thin as in the others of the third group but the epithelium, instead of forming the duct-like condition, is composed of very tall ciliated cells of an actively secretory type arranged in folds which are higher than those in the remainder of this group.

It should be added that three males and three females kept at a temperature of 5°C. were injected similarly to those at the higher temperature. Although two of each sex died before the injections were terminated, the ones remaining evidenced no gonadal stimulation by weight or histological examination.[4]

DISCUSSION

Attempts to determine the environmental factors which are correlated with the sexual periodicity in monoestrous forms have shown conflicting results in some instances and in general have been found to vary with the species under consideration.

In mammals, for example, Johnson, Foster and Coco ('33) noted that in the ground squirrel the period of normal sexual inactivity was considerably shortened under laboratory conditions, whereas Moore and his co-workers ('34) reported that in the male ground squirrel neither light, temperature nor diet would stimulate the germinal epithelium. In the female they found that the combination of cold and darkness over a period of several months resulted in sexual activity at any time of the year. However, in the ferret, Bissonnette

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[4] Male no. 7, body weight 24.5 gm., testes weight (2) 61 mg. Female no. 14, body weight 20.0 gm., ovary weight (2) 25 mg.

('35 b) concluded that the delay of oestrus as obtained in his experiments was directly correlated with the reduction of the duration and intensity of the light periods. In the male ferret he (Bissonnette, '35 a) was able to cause the production of sperm during the period of normal sexual regression by increasing the periods of exposure to light.

That light is an important factor in controlling the periodic sexual activity in birds has been shown by Bissonnette ('33) and others, although the conditions necessary for a stimulatory effect (type of light ray and manner of application) seem to vary with the species. Rowan ('28), on the other hand, considers longer exercise periods, with or without light, to be the direct cause of the sexual cycles in the birds upon which his studies were made.

According to Alexander and Bellerby ('35) the genitalia of the South African clawed toad undergo no degeneration under laboratory conditions if sufficient food is available.

In contrast to these findings, Craig-Bennett ('31) made a rather extensive observational and experimental study on the three-spined stickleback and concluded that there was an absence of a light effect in this species of fish but that the fluctuations in the temperature were of primary importance in respect to the control of the sexual activity.

The results of the present experiment indicate that in the horned lizard the influence of the combination of light, heat and food as applied under the conditions mentioned above is insufficient to produce complete precocious appearance of germinal activity but that these factors added to the injections of anterior pituitary extract cause a development of the gonads and gonoducts comparable to that obtained in normal animals from 1 to 2 months later. Although one group (group II) was kept for a period of time equal to that normally existing between the emergence from hibernation and the onset of germinal activity and under conditions of food and temperature similar to those in the field, the testes were only slightly stimulated and there was no observable effect on the ovaries or the gonoducts. The conditions differed from those of the normal habitat principally in that the animals were not exposed to sunlight. It is possible that this addition is a necessary factor in order for the active condition to be attained.

The numbers of animals which were kept at the low temperature and which showed no response to administration of the extract are too few to warrant any conclusions. However, since the group which was kept at the higher temperature gave a 100% response to injections of the extract, there would seem to be an indication that a combination of the anterior pituitary product and some, or all, of the factors of light, heat and food is necessary to produce the gonadotropic effect.

It seems to the writer from the data at hand that in some species the anterior pituitary may have an inherent cyclic action and that the initiation and maintenance of sexual activity is caused by its action upon a suitable physiological substratum. Also, it seems evident that the cycle may be modified by various environmental conditions to which there is a species difference in sensitivity.

SUMMARY

Male and female horned lizards were kept from the middle of January until the first part of March at a temperature of 35°C., with an oversupply of food available, and under continuous exposure to a 16 candle power electric bulb. The gonadal weights were approximately the same as those of the controls which were kept in hibernation at a temperature of 5°C. Histologically the testes of the animals at the higher temperature showed some stimulation in that the seminiferous tubules contained small lumina as compared to the solid tubules of the controls. However, there was no indication of stimulation of the gonoducts.

Other animals of both sexes kept at the higher temperature, supplied with food and exposed to electric lighting were injected during the last 12 days of the experiment with an extract totaling 3 gm. equivalent (60 R.U.) of the whole anterior pituitary of the hog. In these there was evidenced a stimulation of the gonads and gonoducts comparable to that normally occurring at the early part of the breeding season. In the case of the female the weight rather than the number of the individual eggs was increased. In the male the germinal epithelium was the principal site of the stimulation.

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PLATE

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PLATE 1

EXPLANATION OF FIGURES

1 Photomicrograph of a section of a seminiferous tubule of animal 8, showing the solid nature of the tubule and the absence of mitoses and late spermatogenic stages in an hibernating individual. × 344.

2 Photomicrograph of a section of a seminiferous tubule of animal 1, showing the partial stimulatory effect of light, heat and food as evidenced by the production of a lumen in the tubule. × 344.

3 Photomicrograph of a portion of a section of a seminiferous tubule of animal 2, showing the additional stimulation obtained when an animal which had been kept at a high temperature, exposed to continuous electric light and supplied with food, was injected with the anterior pituitary extract. Note the presence of sperm and mitoses. The letter 'L' indicates the position of the lumen. × 344.

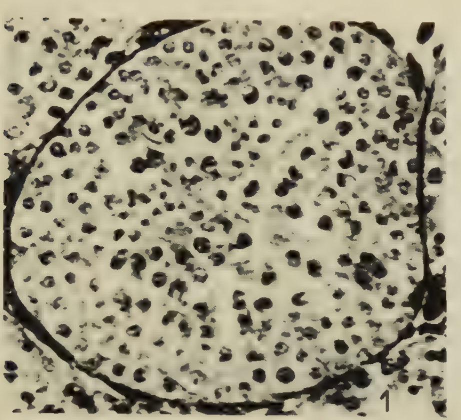
4 Photomicrograph of a portion of a section of the epididymis from animal 2, showing the columnar type of epithelium. × 344.

5 Photomicrograph of a section of the epididymis from animal 8. Note the cuboidal type of epithelium characteristic of the non-injected animals. × 344.

6 Photomicrograph of a longitudinal section of the oviduct from animal 24 to show the thick outer muscular and connective tissue covering and the folded nature of the cuboidal epithelium which is characteristic of the non-injected animals. The letter 'L' indicates the position of the lumen. × 344.

7 Photomicrograph of a longitudinal section of the oviduct from animal 44, illustrating the relatively thin covering and the duct-like nature of the epithelium as obtained following the injection of the gonadotropic extract into females which had been kept at a higher temperature, exposed to continuous electric light and given food. The letter 'L' indicates the position of the lumen. × 344.

8 Photomicrograph of a longitudinal section of the oviduct from animal 42 to show the condition of the epithelium in two females (nos. 40 and 42). The arrow indicates the position of the lumen. The animals had been injected with anterior pituitary extract and kept under the same conditions as animal 44. × 344.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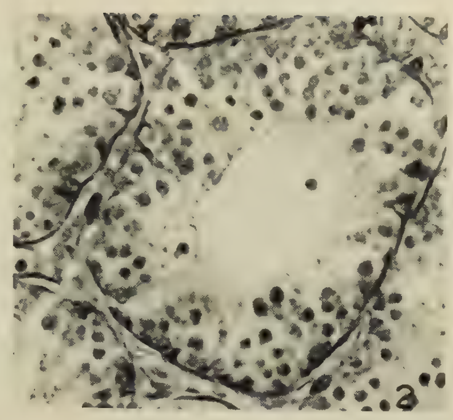
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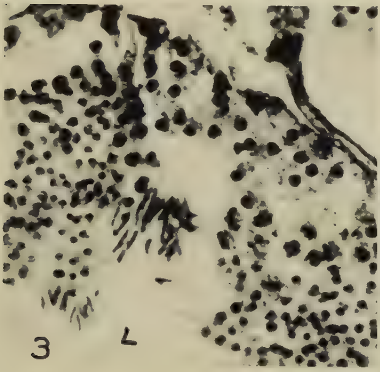
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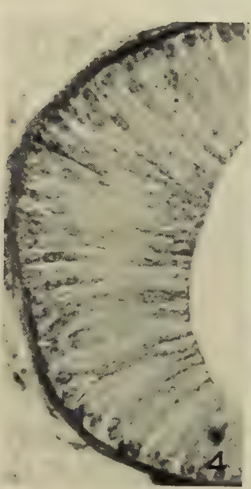
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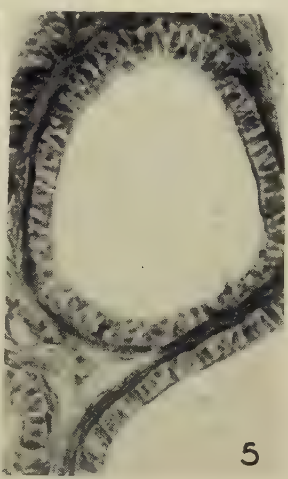
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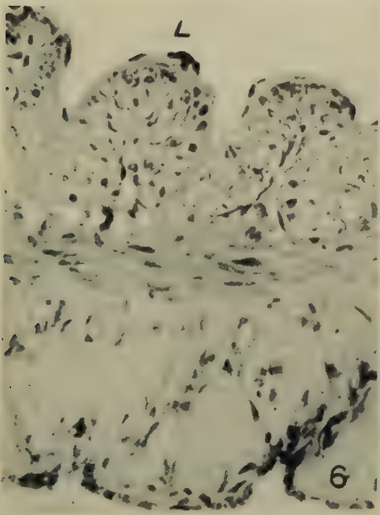
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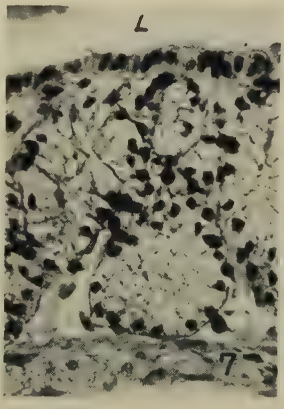
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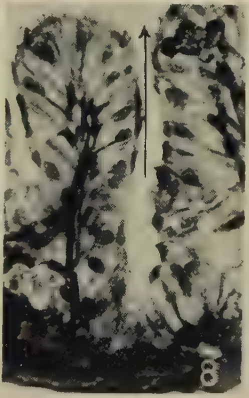
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THE MENINGEAL RELATIONS OF THE HYPOPHYSIS CEREBRI

HENRY G. SCHWARTZ
Department of Anatomy, Harvard Medical School, Boston

TWO TEXT FIGURES AND THREE PLATES (THIRTEEN FIGURES)

A survey of the literature up to 1924 reveals only vague, casual references concerning the arrangement of the leptomeninges in relation to the hypophysis (for earlier literature, see Hughson, '24). From injection methods Hughson concluded that the hypophysis of dogs and cats is completely invested by the subarachnoid space. Atwell ('26) referred to this conception, but did not incorporate it in his diagram of the human pituitary body. In his scheme the gland is completely enveloped by the dura: The arachnoid is pictured as extending only to the pars tuberalis at the neck of the gland. Subsequently Atwell's drawing was modified by Bailey ('28) to show a complete investment of the hypophysis by the leptomeninges, except at the extreme posterior pole where the blood vessels enter the posterior lobe. This has been commonly accepted, although most general texts on anatomy cling to the older illustrations without making any definite statements concerning the exact extent of the arachnoid.

Dyke and Davidoff ('34) studied the normal cerebral structures made visible by encephalography in man and devoted considerable attention to the subarachnoid cisterns. They made no mention of any antero-ventral extension of the cisterna interpeduncularis other than to define its rostral border as the caudal margin of the optic chiasm and the internal carotid arteries. On the other hand, they stated that the cisterna chiasmatis is continuous ventrally with the hypophyseal subarachnoid space. In a brief report, unaccompanied by illustrations, Roussy and Mossinger ('34) concluded that there is not, properly speaking, a perihypophyseal arachnoid space, but that the arachnoid is wedged directly into the continuous fibrous layer which forms the superior boundary of the perihypophyseal network.

The present investigation was prompted by Professor Wislocki who had studied a number of serially sectioned mammalian hypophyses and had been led to doubt the current opinions regarding the meningeal relationships of the hypophysis. In order to define with certainty the anatomical limits of the perihypophyseal subarachnoid space, it was decided to resort to injection of India ink into the leptomeninges. It will be shown in this report that the subarachnoid space is not continuous around the body of the hypophysis of the dog.

MATERIAL AND METHODS

Young dogs (6 to 8 weeks old) were used. Replacement of 1 to 2 cc. of cerebrospinal fluid with a dilute saline solution of India ink was performed by cisternal puncture. The animals were allowed to live for 18 to 24 hours. Under nembutal anesthesia, the tissues were perfused through the heart first with normal saline and then with formalin (1:4). This procedure in itself gave satisfactory fixation. To permit greater infiltration the anterior and posterior poles of the calvarium were removed, and the specimen was placed in formalin for 48 hours. Care being taken to preserve the meninges, the cranial vault was rongeured away, leaving intact the brain surrounded by its membranes and the base of the skull. The base of the skull, with the brain in situ, was then decalcified with trichloracetic acid (5% aqueous solution). The entire specimen was subsequently dehydrated and embedded in celloidin. Sagittal and frontal serial sections were cut, varying in thickness from 30 μ to 200 μ, and stained with carmalum.

OBSERVATIONS

The hypophysis of the dog differs from that of primates in several respects. Instead of hanging perpendicularly from the diencephalon, it is directed posteriorly, so that its long axis comes to lie almost parallel with the floor of the brain. To accomodate this structure, the rostro-caudal extent of which is greater than its depth, the dorsal surface of the sphenoid bone shows only incomplete sellar formation. The dorsum sellae and the posterior clinoid processes are well developed. Anteriorly, however, there is only a slight bony elevation, without true clinoid differentiation. The body of the gland thus lies only partially in a shell of bone.

As noted by Stendell ('14), the spatial relationship between the epithelial and neural constituents in the dog differs from that found in primates. The pars distalis surrounds the pars neuralis. The pars intermedia, likewise, may be found around the periphery of the pars neuralis. The infundibular process is enveloped by the collar-like pars tuberalis, which is reflected onto the diencephalon. The tuberal projection extends farther anteriorly than it does posteriorly.

In both sagittal and frontal series, it was found that the injected ink spread effectively over the entire basal surface of the brain, clearly outlining the subarachnoid space even into the depths of the sulci.

In plate 1 are shown photomicrographs of sagittal sections through the brain and base of the skull. The distinctive features of the hypophysis and sphenoid bone are apparent. Because of shrinkage of the body of the hypophysis away from the sella, the normal relationship of the dura is somewhat distorted. The dura passes over the posterior clinoid process to the superior-posterior portion of the pars distalis, just like the diaphragma sellae of man. As it meets the pars distalis, it becomes directly continuous with the capsule of the gland, and, under high power (fig. 7), can be traced almost to the pars tuberalis above. Below, the dura normally blends intimately with the capsule and bony periosteum, extending around the pars distalis in this fashion to the pars tuberalis anteriorly. It then continues forward to the rudimentary anterior clinoid process, from which point it joins the dura covering the surface of the brain. Anteriorly and posteriorly the dura splits to form the communicating intercavernous sinuses. Although the photographs apparently show a wide space separating the gland from the dura anteriorly and inferiorly, study of sections under high magnification leads to the conclusion that this is due solely to shrinkage, since numerous lacy strands can be seen traversing this space, connecting the hypophysis with the dura, from which it has been pulled away. Further corroboration of the intimate envelopment of the gland by the dura is found in frontal sections where, fortunately, shrinkage is minimal (plate 3). Indeed, sections through the body of the gland fail to reveal any discrete line of demarcation between capsule, dura and periosteum.

The ink-containing subarachnoid space, however, does not invest the body of the gland. In a mid-sagittal section (fig. 4), the space extends anteriorly over the pars tuberalis to the proximal end of the pars distalis. At this point, under low magnification, it appears to pass almost into the capsule of the hypophysis. Under high magnification (fig. 8), however, the arachnoid obviously ends in a blunt, rounded process made up of clusters of cells distinctly separate from the capsule. The increased aggregation of cells at this point resembles a similar arrangement of the spinal arachnoid at the site of emergence of the spinal nerve roots (Elman, '23). Posteriorly, the space does not extend quite so far down onto the hypophysis as it does anteriorly. It ends in a rather large bulbous cul-de-sac at the mid zone of the pars tuberalis (figs. 3 to 6). This is especially well shown in figure 9, where, under high magnification, the limits of the cul-de-sac are clearly defined by collections of ink particles. It should be noted that no injection material is found surrounding the body of the gland beyond the limits at which the arachnoid has been shown to end.

Paramedian sections (figs. 3, 5 and 6) show a recession of the anterior extension of the arachnoid. On either side of the midline it passes laterally and proximally, receding from the gland to the posterior margin of the pars tuberalis where it becomes continuous with the rostral portion of the cisterna interpeduncularis. Figure 1 shows the course taken by the subarachnoid space around the upper portion of the hypophysis.

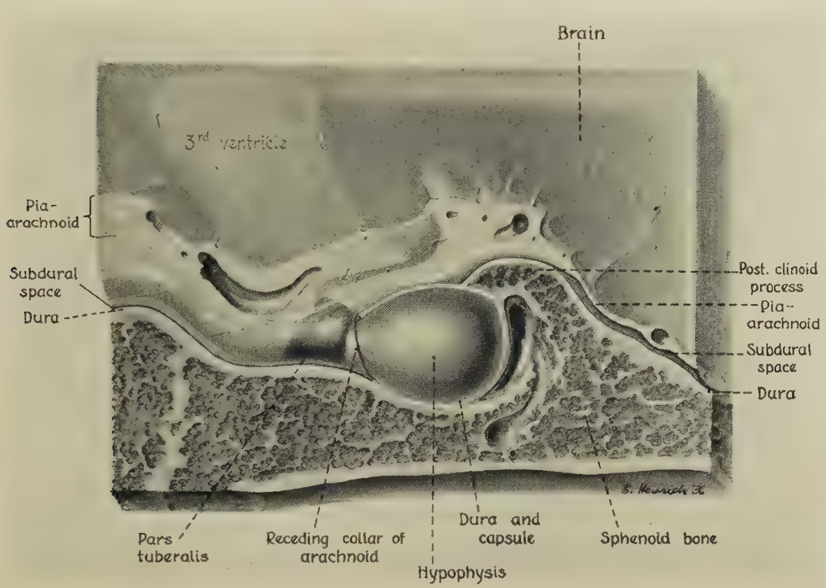
Brain
3rd ventricle
Pia-arachnoid
Subdural space
Dura
Post. clinoid process
Pia-arachnoid
Subdural space
Dura
Pars tuberalis
Receding collar of arachnoid
Hypophysis
Dura and capsule
Sphenoid bone

Fig. 1 Semi-schematic drawing of the hypophysis in situ, viewed from the side. Anteriorly the arachnoid membrane extends over the pars tuberalis to the proximal end of the pars distalis. From this point it recedes around the neck of the gland to the pars tuberalis posteriorly. There is no prolongation of the arachnoid into the sella. The body of the gland is enveloped by the dura mater, which extends throughout the sella.

Since the arachnoid space extends farther down anteriorly than it does posteriorly, it is readily understood how a frontal section directed slightly obliquely may show the anterior portion of the pars distalis surrounded laterally and ventrally by the ink-containing space (fig. 10). In sections farther posterior, however, the recession of the arachnoid space and the consequent absence of any injected material around the ventral and ventro-lateral surfaces of the main mass of the pituitary are clearly demonstrable (figs. 11, 12 and 13).

The disposition of the dura also supports our contention that the subarachnoid space does not envelope the body of the gland. In frontal sections (figs. 11 and 12), the dura is seen passing directly over from the sides of the sella to the hypophysis. Here it splits into two layers: The upper layer passes up to the pars tuberalis; the lower layer goes down into the depths of the sella around the body of the hypophysis. Both upper and lower laminae become insensibly blended with

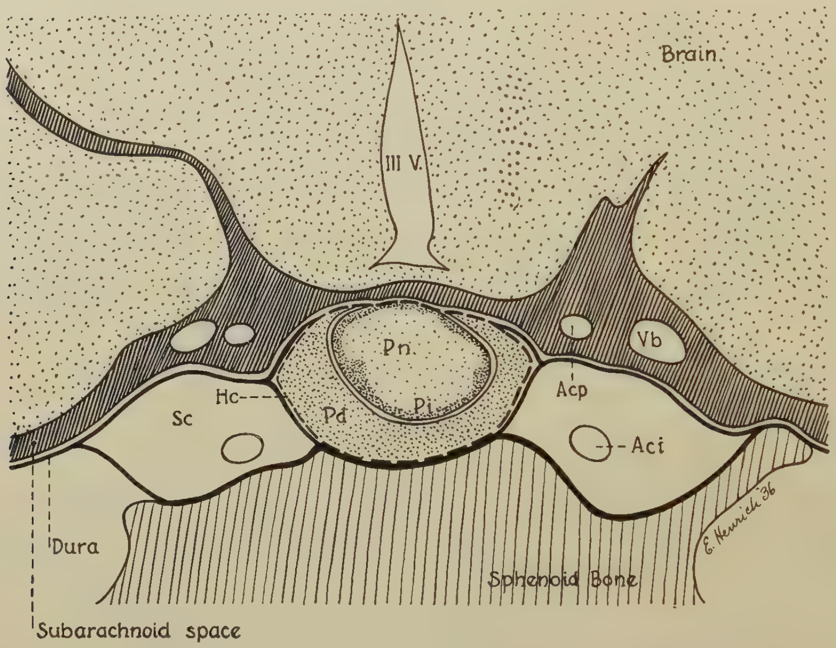

Fig. 2 Diagrammatic representation of a frontal section through the body of the hypophysis, just posterior to the infundibulum. The arachnoid passes above, taking no part in the envelopment of the body of the gland. The dura mater splits into two laminae, which blend with the hypophyseal capsule (Hc) around the circumference of the gland. III V, third ventricle; Aci, internal carotid artery; Acp, posterior communicating artery; Sc, cavernous sinus; Vb, basilar vein; Pd, pars distalis; Pi, pars intermedia; Pn, pars neuralis.

the hypophyseal capsule. Under such conditions it is difficult to suppose that the overlying subarachnoid space may pass the barrier afforded by the upper dural lamina (figs. 2, 14 and 15).

DISCUSSION

It has been suggested (Hughson, '24) that, although a completely enveloping subarachnoid space may not be found under normal manometric conditions in dogs and cats, it may be demonstrated by cisternal injection of Prussian blue precipitating reagents under abnormal circumstances, namely: 1) When the cerebrospinal fluid pressure is reduced to below zero, by intravenous injection of hypertonic saline; or 2) when it is raised by injection of the foreign salts under an increased pressure of 50 to 80 mm. of the fluid. The present group of experiments on dogs was carried out under normal pressure conditions by replacement of cerebrospinal fluid with India ink. The sections have shown with sufficient clarity the exact histological extent of the subarachnoid space, and warrant the conclusion that even under abnormal pressures, a true subarachnoid space could not be present around the body of the hypophysis.

The presence of Prussian blue granules completely surrounding the hypophysis, in Hughson's experiments, does not necessarily prove that the subarachnoid space extends around the organ. In view of the present observations it follows that the Prussian blue observed by Hughson lies within the interstices between the dura and hypophyseal capsule. There are several conceivable explanations to account for the presence of the color in this situation.

1. The first of these explanations concerns a consideration of the pathways of escape of the cerebrospinal fluid under normal and abnormal pressures. There can be no doubt that the course of true solutions (such as the foreign salts of the Prussian blue reaction), following injection into the subarachnoid space, demonstrates not the limits of the space alone (as does particulate matter), but the pathways of absorption of the cerebrospinal fluid. This was pointed out by Elman

('23), in connection with his investigations of the spinal arachnoid granulations. He noted that injections of India ink satisfactorily demonstrated the anatomical limits of the subarachnoid space, but that such injections could not be used in tracing functional pathways of absorption because the intact arachnoid mesothelium is impermeable to carbon particles. To investigate the existence of physiological channels of absorption he therefore used a true solution of Prussian blue reagents. Elman was able to trace the granules from the subarachnoid angle at the point of emergence of the nerve root, and found them to be abundant in the surrounding dural tissue. No granules were found inside the nerve fibers or in the so-called perineural space. The granules were found to make their way through the dura to the veins outside the cord. An analogous situation may well be considered in the case of the hypophysis. The gland itself may be compared with the emerging spinal nerve. The arachnoidal cell clusters which appear at the spinal subarachnoid angle are quite analogous to the cell tuftings of the arachnoid granulations of the cerebrum. Weed ('14) has noted the presence of arachnoid villi in connection with the cavernous sinuses, and they have been seen in our sections. The presence of villi in the neighborhood of the hypophysis suggests that some absorption of fluid takes place in this area. It would not be surprising therefore to find some blue granules in the dura which closely envelopes the pituitary in this region, especially under the altered pressure relationships that obtained in Hughson's experiments.

2. A second conceivable explanation for the presence of Prussian blue precipitate around the hypophysis in Hughson's experiments involves a consideration of the possibility of rupture of the leptomeninges in this locality. As the arachnoid approaches the sella, it becomes partially fixed by arachnoidal villi to the dura overlying the cavernous sinuses. This in itself might be a predisposing factor for rupture of the arachnoid in this region under abnormal fluid pressure conditions. Our sections show, moreover, that on either side

of the hypophysis the posterior communicating artery passes from the intradural internal carotid artery into the cisterna interpeduncularis (fig. 10); it then courses upward toward the brain where it joins the posterior cerebral artery. A weak area accordingly results where the posterior communicating artery passes through the arachnoid, since here is a point at which a delicate membrane, elsewhere relatively mobile, is fixed by the adventitia of the comparatively immobile artery. If alteration of pressure is sufficiently abnormal to cause rupture, it should be expected from the anatomical relationships in this region that a break would occur in the vicinity of the hypophysis.

3. It is further conceivable, although less likely, that the Prussian blue gains access in some measure to the circumference of the hypophysis as a result of post-mortem artefact, especially if it is attempted at autopsy to dissect the gland and its meninges from the sella. Indeed, the pituitary of the dog, as has been demonstrated in the present study, is so intimately attached by its capsule to the dura lining the sella, that dissection of the organ without the production of serious artefacts is almost impossible. Tension on the dura, through manipulation of the sphenoid bone, is readily transmitted to the body of the hypophysis and the collar of the arachnoid around the pars tuberalis. To preclude such a possibility, our specimens were fixed, sectioned, and stained with the partially encircling sphenoid bone intact. We attribute the difference between our conclusions and the current opinions on the disposition of the perihypophyseal meninges largely to the fact that in our study the pituitary was sectioned in situ.

Our results have shown that in the dog there is no demonstrable subarachnoid space around the body of the hypophysis, and that the dura is adherent to the hypophyseal capsule. The question arises as to whether the findings in the dog apply equally to other mammals. Material at our disposal of rat, rabbit, cat and monkey indicates that the same fundamental conditions exist in those forms as well.

SUMMARY

1. Observations have been made on the normal relationships between the dog's hypophysis, the meninges and the sphenoid bone.

2. By replacement of a small amount of cerebrospinal fluid with India ink, it has been observed that the subarachnoid space does not invest the body of the hypophysis. Anteriorly the cisterna chiasmatis extends only as far as the proximal end of the pars distalis. From this point the subarachnoid space courses around the neck of the gland to the pars tuberalis posteriorly, joining the rostral boundary of the cisterna interpeduncularis.

3. The dura extends throughout the sella and blends imperceptibly with the hypophyseal capsule, so that no true subdural space is apparent in this region.

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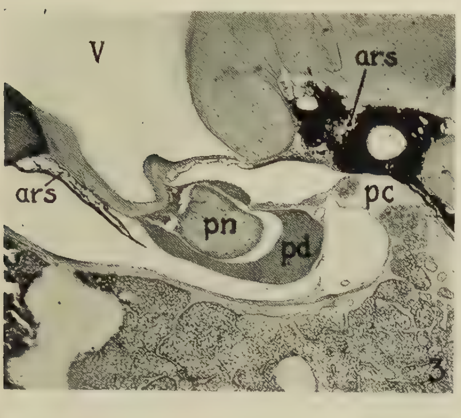
PLATES

PLATE 1

EXPLANATION OF FIGURES

3, 4, 5, 6 Photomicrographs of sagittal sections of the hypophysis in situ. Figures 3, 5 and 6 are paramedian, and figure 4 is a median section. The subarachnoid space (ars) is filled with India ink. Note numerous lacy strands between dura and hypophyseal capsule (dc). dm, dura mater; pc, posterior clinoid process; pd, pars distalis; pi, pars intermedia; pn, pars neuralis; pt, pars tuberalis; sic, intercavernous sinus; sph, sphenoid bone; ssph, sphenoidal sinus; v, third ventricle. Magnification × 6.

7 Photomicrograph of sagittal section showing extension of the dura from the posterior clinoid process along the posterior border of the pars distalis, toward the pars tuberalis (pt). dc, blending of dura and hypophyseal capsule. Magnification × 145.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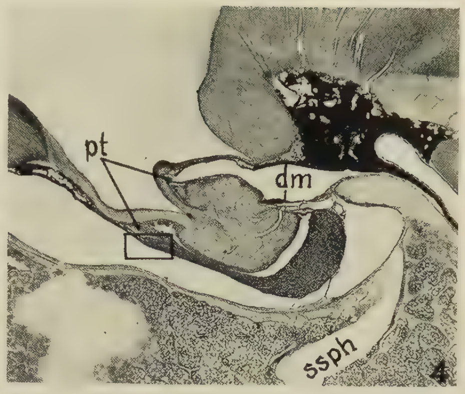
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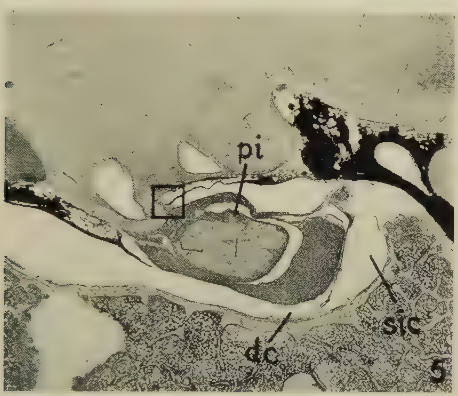
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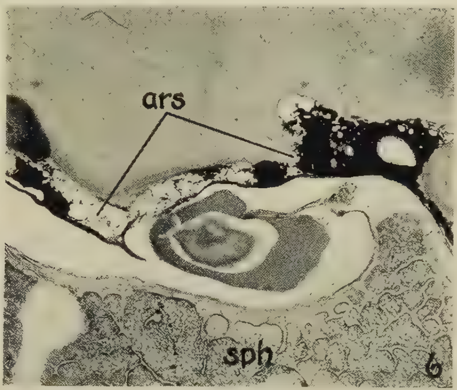
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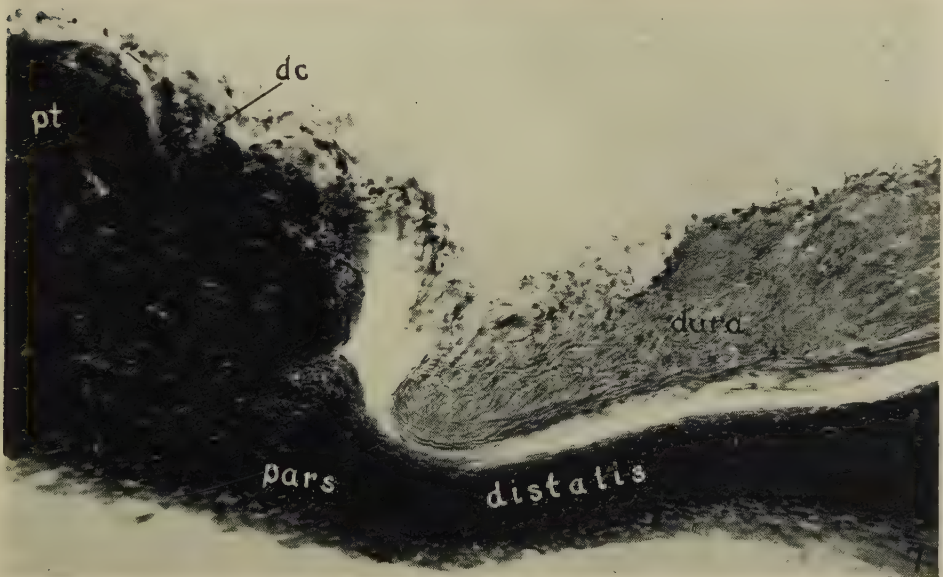
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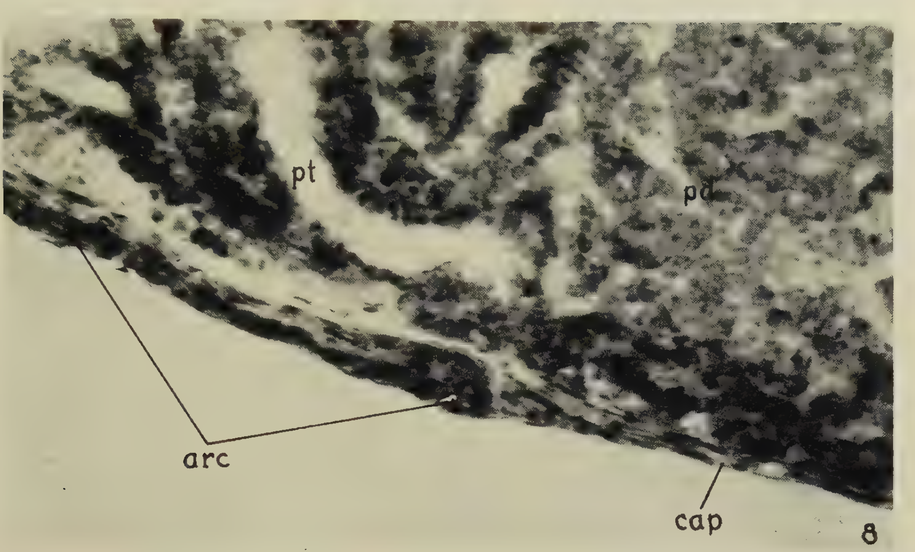
PLATE 2

EXPLANATION OF FIGURES

8 Photomicrograph of region indicated within the square in figure 4, showing the arachnoid ending in a blunt cluster of cells (arc) clearly demarcated from the hypophyseal capsule (cap). pd, pars distalis; pt, pars tuberalis. Magnification × 335.

9 Photomicrograph of area indicated within the square in figure 5. The arachnoid membrane (ar) is well circumscribed as shown by the accumulations of India ink. ars, subarachnoid space; inf, infundibular process. Magnification × 145.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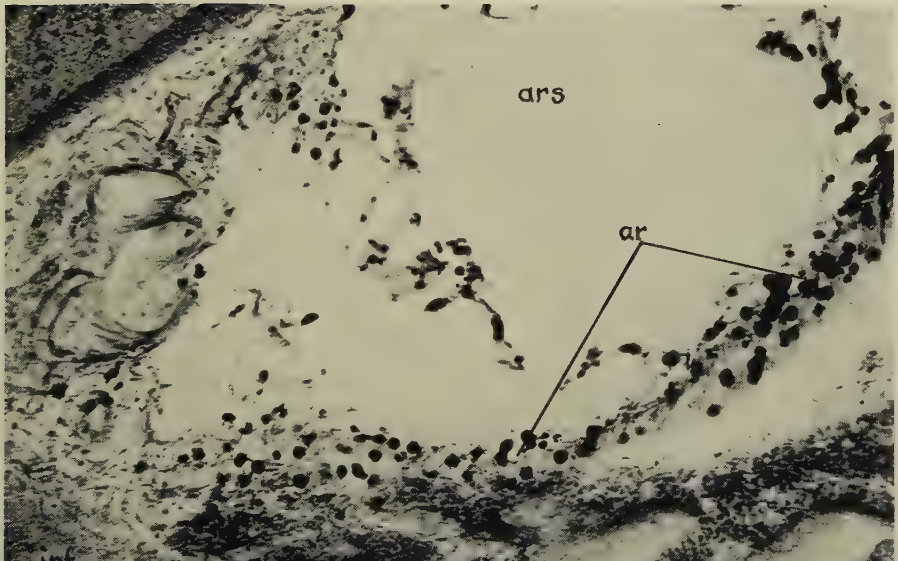
ars
ar
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pars tuberalis

9

PLATE 3

EXPLANATION OF FIGURES

10, 11, 12, 13 Photomicrographs of frontal sections through the hypophysis in situ. Figure 10, through the most anterior portion of the pars distalis (pd). The subarachnoid space passes beneath the pars distalis at this place as shown by the distribution of the ink. The posterior communicating artery (pca) passes from the internal carotid artery (ica) through the arachnoid. In figures 11, 12, 13, the recession of arachnoid posteriorly is clearly demonstrated. Note the intimate relationship of the periosteum, dura and hypophyseal capsule (pdc). pn, pars neuralis; scav, cavernous sinus; sph, sphenoid bone. Magnification × 6.

14 Photomicrograph of the area indicated within the square in figure 12, showing splitting of the dura mater (dm) into two laminae which become continuous with the hypophyseal capsule. Except within the subarachnoid space (ars) no ink particles are visible. scav, cavernous sinus; pdc, periosteum, dura and hypophyseal capsule. Magnification × 31.

15 Photomicrograph showing splitting and reflection of the dura mater (dm) into two laminae. ars, subarachnoid space. Magnification × 31.

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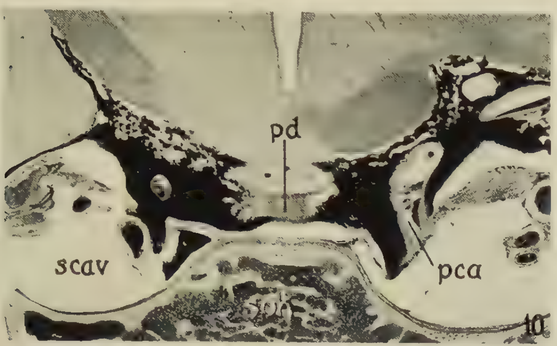
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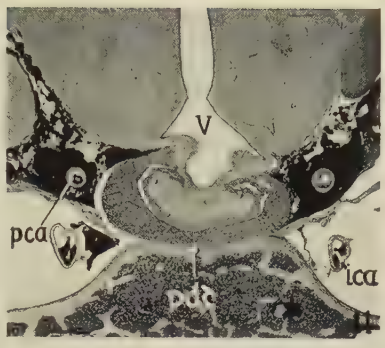
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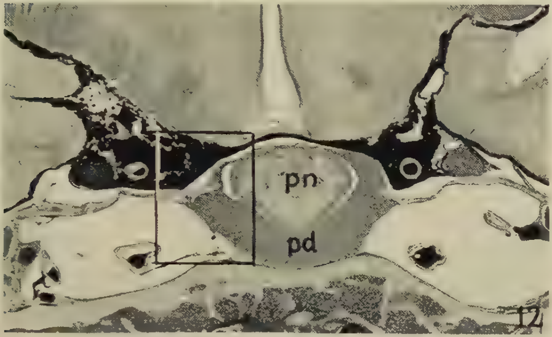

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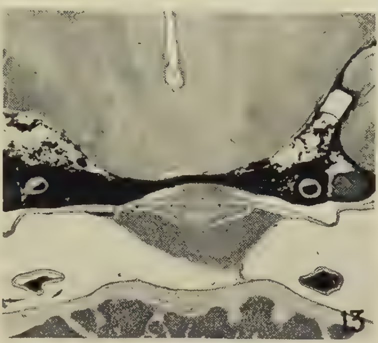

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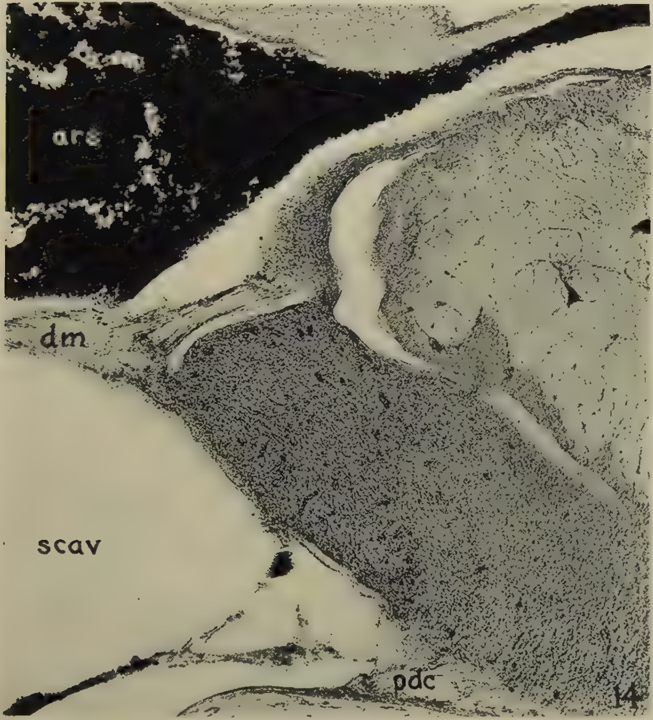
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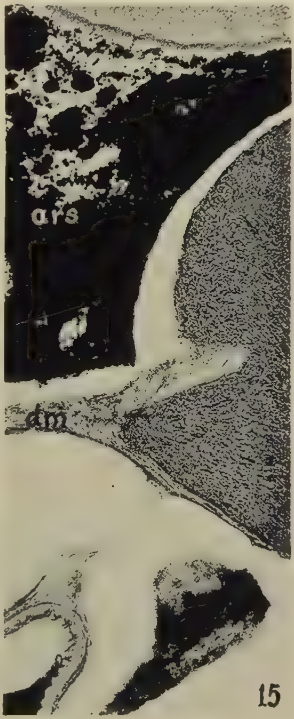

15

THE CYTOLOGY OF SNAKE THYROIDS FOLLOWING HYPOPHYSECTOMY, ACTIVATION AND ULTRACENTRIFUGING

HALCYON W. HELLBAUM
Department of Zoölogy, University of Wisconsin

TWO PLATES (THIRTY-THREE FIGURES)

A comprehensive and thorough study of the cytology of normal and activated thyroid glands has recently been made by Severinghaus ('33). Employing the activated thyroid of the duck, sheep, guinea pig and monkey he has focussed attention upon the secretion of the gland. In the present study, emphasis has been placed upon cytological changes of the gland induced by hypophysectomy, activation following pituitary administration, and ultracentrifuging to obtain the relative specific gravities of the various cell elements. The garter snake has been chosen for these experiments because of the ease with which the pituitary gland can be removed, thus allowing the use of a large number of animals. In addition, snake thyroids are readily activated by anterior pituitary extracts of sheep. Furthermore, such glands lend themselves particularly well to a study of the intracellular constituents, and especially of the Golgi apparatus.

Since it has not been the object of this paper to discuss the mitochondria, description of these cytological structures will be omitted.

It is a pleasure to express my appreciation to Prof. M. F. Guyer for his generous and painstaking cooperation and continued interest throughout the work; and to Dr. W. H. Schaefer, who demonstrated his method of hypophysectomy.

I wish also to thank Dr. Pearl E. Claus, for her helpful directing in centrifuging the thyroids; and to Mr. E. J. Dornfeld, for his invaluable suggestions on some of the cytological technique.

METHODS AND MATERIALS

The materials used for this study consisted of the thyroids of more than 300 snakes. The two species of common garter snakes used were collected in the vicinity of Madison, Wisconsin. They were marked by removing certain ventral scales of the tail region according to the method described by Blanchard and Finister ('33). More than 100 of these were hypophysectomized according to the method worked out and described by Schaefer (unpublished), as follows:

> Animals to be hypophysectomized were anesthetized with ether, and placed under an ordinary dissecting binocular. The roof of the mouth was exposed to view by pinning the upper jaw to a soft board, and permitting the floor of the mouth to rest against two needles placed in a perpendicular position. A longitudinal incision about 3 mm. in length was made in the oral mucosa under semi-sterile conditions. Muscle tissue was scraped aside exposing the ventral surface of the sphenoid bone opposite to the hypophysis. By inserting a sharp pointed scalpel at several places around this region, care being taken to prevent brain injury, the small disc of bone so loosened was lifted away exposing the pituitary body. A small hook made from an insect pin was used to remove only the anterior lobe or the entire body. To permit free drainage in case the area became infected, the bony disc was usually not replaced. Healing, in general, was rapid. Control operations were made in a similar manner, except that the pituitary body was not removed. The operation is a comparatively simple one, first, because the mouth can be widely opened and, second, because of the absence of a nasopharynx.

This technique was used except that in actual extirpation, a minute glass cannula was used and the anterior lobe or the whole gland was easily sucked out. In no case was death caused by the operation.

In order to obtain the regressive changes in the thyroid, one series of forty-six snakes was hypophysectomized; two were autopsied each day for 14 days and from then on at intervals of 16, 20, 25, 28, 30, 32, 39, 49 and 80 days. Normal animals were killed throughout the experiments as controls. The thyroids were removed and fixed immediately according to methods described below.

Another series of fifty-seven snakes, hypophysectomized for 30 days, was given subcutaneous daily injections of 0.1 gm. equivalents of anterior pituitary extract of sheep in 0.1 cc. of distilled water. Three snakes were killed daily for 15 days, after which the intervals between killings were lengthened to 18, 21, 26 and 30 days.

Six normal and two 80-day hypophysectomized animals were treated with a thyrotropic fraction of sheep anterior pituitary (figs. 3, 4 and 6), prepared according to the method of Greep ('35), and the results were comparable to those receiving the non-fractionated pituitary extract prepared as described by Fevold, et al. ('33).

Of nine non-hypophysectomized snakes, injected with anterior pituitary extract daily for 6 days, the thyroids of three were ultracentrifuged for 20 minutes, three for 60 minutes and the remaining three served as controls. The glands were removed and placed in isotonic Locke's solution in the metal rotor of the ultracentrifuge designed by Beams and Weed ('31, '33). A centrifugal force of about 400,000 times that of gravity, obtained with an air pressure of about 100 pounds, was exerted on the thyroids for the above-mentioned lengths of time.

In preliminary work on techniques, the best fixative for the Golgi apparatus in the thyroids of these snakes proved to be the Severinghaus modification of Champy. Bouin, Bensley, and Regaud fixatives were also found satisfactory for general cellular and nuclear study. The above fixatives were used exclusively on all the experimental material. Osmication for 4 days was found to be sufficient for impregnation of the Golgi, with destaining unnecessary in most cases. When destaining was necessary, a weak solution of hydrogen peroxide was used. The new dioxan method of dehydration was utilized (for a description of the procedure, see Guyer, '36). All tissues were embedded in paraffin and cut 4 μ in thickness except for several which were cut at 2 μ. Champy was seldom followed by a counter stain, although on some, acid fuchsin and methyl green were used. These stains also followed the Bensley fixative. Iron hematoxylin was used after all three fixatives, while Delafield's hematoxylin and eosin were employed after Bouin only.

OBSERVATIONS

The snake thyroid—normal control. The reader is referred to a paper by Atwood ('18) for a gross anatomical study of the viscera of the garter snake. To summarize briefly, the thyroid is a single rounded body lying just anterior to the heart, supplied with blood by the right and left carotid arteries and drained of blood by a branch to the right jugular vein. The gross histological picture is a close aggregation of follicles of variable size. The epithelium surrounding the follicles varies slightly from low columnar to cuboidal. It will be observed from figures 1, 2 and 14, that the nucleus is normally nearer the base of the cell and the Golgi apparatus is always found between the nucleus and the lumen and most frequently fairly close to the nucleus. From a polar view, the network frequently appears circular (fig. 15). The nucleus is somewhat rounded but may vary slightly with the variation of the height of the cell. The nucleolus stains deeply with Delafield's hematoxylin, and the chromatin is clumped on the linin network. The Langendorff colloid cells stain darkly with osmic acid leaving the nucleus large and clear (fig. 23). These cells are sparsely scattered in the epithelium of the follicles. Intracellular colloid droplets are seen throughout the cell.

The activated snake thyroid. The thyroid cells of animals stimulated for 6 days with anterior pituitary (figs. 4 and 5) become columnar extending into the lumen which appears smaller. The cytoplasm becomes highly vacuolated with lightly staining colloid. The nucleus does not seem to be altered materially (fig. 17). The Golgi apparatus appears as a looser network and so takes up more space in the cell, and comes to rest nearer the lumen, away from the nucleus. The Langendorff cells also increase in size, and are found more frequently than in the normal gland (figs. 4 and 29). Stages of further degeneration of these cells are seen in figures 30 and 31.

The snake thyroid after hypophysectomy. Twenty-eight days after hypophysectomy, large colloid-filled follicles are seen (fig. 7). The epithelial cells lining these follicles become very much flattened (figs. 8 and 32). The nucleus takes a deeper stain and lies very close to the basal membrane. In polar view (fig. 33), the nucleus appears round and larger than that of a normal cell (fig. 19), which is probably due to a pressure exerted from within by the enlargement of the whole follicle, resulting in this flattening of both the cell and the nucleus. The nucleus appears to stain less deeply in polar view. The Golgi bodies are pycnotic and consist of small definite clumped masses (fig. 27). Usually they are found at the apical pole (fig. 24), but may frequently be seen at the side of the nucleus (fig. 25), and sometimes, though more rarely, may be found at the basal pole (fig. 26).

Following hypophysectomy, there is a gradual change from the normal to this non-active condition. In this regressive series, the greatest change from normal to the non-active type occurs within the first 10 days. From then on, the change is much more gradual. There seems to be very little difference in appearance of 80-day hypophysectomized from that of the 28-day hypophysectomized animal.

The thyroids from the 30-day hypophysectomized snakes that were activated with anterior pituitary showed a rapid return to normal. After four or five daily injections the glands were similar to those of the normal and exhibited marked stimulation after ten injections (fig. 6). After fifteen injections, the number of Langendorff cells were extremely numerous (fig. 11), some follicles showing as many as half the number of the chief cells. After twenty-one injections, intrafollicular colloid had practically disappeared and the lumen been almost obliterated by the hyperplasia of the epithelial cells (fig. 10).

A study of the sections of the thyroids of hypophysectomized snakes which have been activated shows first a heightening of the epithelium and then, with continued treatment, a folding of the epithelium which gives the gland a different appearance (figs. 6 and 10).

Ultracentrifuged thyroids of activated snakes. After ultracentrifuging, the whole thyroid is greatly flattened in the direction of the centrifugal pole (fig. 12). Histological examination shows a flattening of the lumen of each follicle and some shortening of the cells at the centrifugal pole of the follicle. On the other hand, a slight elongation of the cells at the centripetal pole is apparent. The most striking changes have occurred after 60 minutes of centrifuging. In these thyroids, fixed with Champy, the cells on the centripetal side of each follicle show the Golgi apparatus completely reversed from the normal apical position and the nucleus forced in the opposite direction toward the apical portion of the cell, e.g., in the direction of the centrifugal pole (fig. 22). The intracellular colloid droplets are also displaced, lying between the nucleus and the Golgi apparatus.

After Bouin's fixative the nucleus is found in the extreme apical pole (figs. 9 and 18), and the cytoplasm shows a pronounced stratification of the materials. The denser cytoplasm is forced toward the centrifugal side leaving large intracellular vacuoles toward the centripetal pole. An intranuclear stratification is also seen. The chromatin is displaced to the centrifugal pole along with the intranuclear reticulum and the nucleolus. The nuclear membrane elongates and at the centripetal pole, strands of linin network and karyoplasm remain in connection with it (figs. 9, 20 and 28). Such nuclear distortion is similar to that demonstrated in the rat by Dornfeld ('36), and in the centrifuged pituitary of the same animal by Guyer and Claus ('36).

The cells on the centrifugal side of each follicle show merely an accentuated stratification of the normal relations. The nucleus is forced more nearly to the base of the cell, the Golgi moves to the extreme apical pole and the intracellular colloid droplets lie between the Golgi and the nucleus.

After centrifuging 20 minutes, displacements intermediate between the uncentrifuged and centrifuged extremes prevail (fig. 21). In many cells following centrifuging for this shorter time, the Golgi apparatus is stretched along the side of cell in its movement toward the base, and the nucleus is midway toward the apical pole. The intracellular colloid droplets have not become so highly stratified.

DISCUSSION

The snake offered favorable material for cytological study of the thyroid. They are plentiful in the vicinity of Madison, Wisconsin, are easily kept in the laboratory in small cages and necessitate no feeding because of the fat storing bodies. The pituitary gland is easily removed and the thyroid readily responds to various experimental changes. Further, their normal microscopic appearance is similar to those of ''all animals from fish to man'' (Marine, '32).

In snakes, injected with anterior pituitary containing the thyrotropic horomone, the essential cytological observations recorded by Severinghaus ('33) have been confirmed. Accordingly, it is our purpose in this part of the study simply to reemphasize the prevailing interpretations (Severinghaus, '33; Bensley, '16; and Uhlenhuth, '24, '34) of the appearance of the thyroid following activation. As indicated by the work of these investigators, other animals show the same increase of height of the cells after pituitary activation. The Golgi bodies also become more conspicuous and move slightly toward the apical pole from their proximity with the nucleus. No change in the nucleus was detected, and most of the stored colloid was discharged. There is an increase in the number of the exhausted Langendorff cells, and some appear to be in the process of complete disappearance (figs. 4 and 29 to 31).

There is every indication that the activity of the thyroid is definitely controlled by the anterior pituitary. Schaefer (unpublished), showed that no change from that of the normal could be detected after removal of the posterior lobe only. Furthermore, it has been found that the histology of the thyroid, after complete hypophysectomy, did not differ from that following removal of anterior lobe only.

The most significant results of these studies are from the hypophysectomized animals. Smith ('30), studying thyroids of hypophysectomized rats, found that there was a definite decrease in weight of the thyroids, and a marked 'atrophy' of the epithelium which "is not associated with a distention of the vesicles, for the colloid content is not markedly changed." He found the presence of approximately the same amount of colloid in the operated as in the control animals. In this investigation involving snakes, the size of the follicles are increased after hypophysectomy (fig. 7), and more intrafollicular stored colloid is apparent. Although no weights have been taken on these thyroids, the histological picture is definitely different from that shown by Smith. This difference may be explained on the basis that snakes, cold-blooded animals, can live a comparatively inactive existence after pituitary ablation, during which time, more colloid is stored in the follicle. A polar view of these cells shows that the nucleus as well as the cell body is even larger in diameter than those of the normal or activated glands. This indicates that the actual cell size of the glands following hypophysectomy is not altered to the extent that seems apparent on first study. With the flattening of the cells, the Golgi bodies are forced slightly to one side of the nucleus, and in some cases are found at the base of the cell.

When 30-day hypophysectomized snakes are activated by anterior pituitary, the return to normal is very rapid, and may be exaggerated with extended treatment. Many of the thyroids activated more than 15 days showed numerous Langendorff cells, which further suports the idea that these cells may be degenerating chief cells, the secretions of which, have been emptied into the follicles. After eighteen injections or more, the cells do not show a noticeable change in height, but the gland as a whole takes on a different aspect. According to Severinghaus ('33), the folds almost obliterating the lumen (fig. 10), are due to proliferation of the follicular epithelium.

In the activated centrifuged glands, the stratification of the intracellular and the intranuclear materials show a definite difference in specific gravities. That redistribution of materials does not significantly alter the vitality of certain glandular cells is shown by the fact that transplantation of rat pituitary tissue centrifuged for 20 minutes results in continued growth and activity (Guyer and Claus, unpublished). These authors have further observed that the cells, when left in Locke's solution for a short time after centrifuging, will reassume their normal appearance due to their own natural elasticity. An indication of this is apparent in figures 18 and 22, where one thyroid, after centrifuging, was cut in two pieces and 1 part was fixed with Bouin (a quick penetrant) while the other was fixed in Champy (a slower penetrant). It will be observed that the nuclei, when fixed quickly after centrifuging are found at the apical pole, while those fixed with the slower penetrant, have moved back slightly toward the centripetal pole. The stratification indicates that the specific gravities vary from highest to lowest in the following order: Nucleus, cytoplasm containing colloid vacuoles, denser cytoplasm and Golgi apparatus. In snakes then, the Golgi substance is the lightest in weight. This differs from the conditions that prevail in some other tissues such as the adrenal (Dornfeld, '36) where the secretion droplets are shown to be the lightest. In no instance was there any Golgi material passed into the lumen as found in uterine gland of guinea pig by Beams and King ('34). However, a number of nuclei were forced through the plasma membrane into the colloid filled lumen. This would substantiate the belief that the nucleus has the greatest specific gravity of the various cell structures.

SUMMARY

The thyroids of 334 snakes (Thamnophis radix and T. sirtalis) were used in this study. Normal snake thyroids, are similar to those of other animals in that they have low columnar to cuboidal type of epithelium, apically placed Golgi network, and basally located nuclei. After six daily injections of anterior pituitary the cells become highly columnar and the Golgi apparatus enlarges into a prominent network. Langendorff cells are more numerous, and the follicles are relatively smaller in size than those of the controls. One series of forty-six hypophysectomized snakes autopsied at various times showed regressive changes in that the epithelium became more flattened, the Golgi material more pycnotic, and the follicles enlarged. Another series of fifty-seven snakes hypophysectomized for 30 days was injected daily with anterior pituitary. After 4 or 5 days of such replacement therapy, the epithelium was brought back to normal, and after 10 days, showed extreme exaggeration. Longer periods of injections changed the histological picture by increasing the number of Langendorff cells, and by causing the epithelium to almost obliterate the lumen. After 6 days of activation the thyroids were ultracentrifuged for 20 and 60 minutes. Stratification showed the order of specific gravities from highest to lowest to be as follows: nucleus, cytoplasm with colloid vacuoles, denser cytoplasm and Golgi apparatus.

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PLATE 1

EXPLANATION OF FIGURES

Figures in plate 1 are untouched photomicrographs, and all figures are from thyroids of snake. Arrows indicate the direction of the centrifugal force in the centrifuged thyroids.

1 Normal control, no. 42, showing apically arranged Golgi bodies and normal-sized follicles. Champy unstained. × 125.

2 Normal, no. 56, illustrating normal cuboidal epithelial cells. Bouin, Delafield-eosin stained. × 537.

3 Activated 6 days with thyrotropic extract, no. 197, exhibiting decreased size of lumen, increased height of epithelium, Langendorff cells, Lc. Champy unstained. × 125.

4 High power of figure 3. Langendorff cells, Lc; Golgi bodies, G. × 537.

5 Activated 6 days with unfractionated anterior pituitary, no. 331, indicating nuclear position. Bouin, Delafield-eosin stained. × 537.

6 Hypophysectomized for 80 days, injected 10 days with anterior pituitary, no. 166. Notice the decrease in the size of the lumina and the folding of the epithelium. Bouin, Delafield-eosin stained. × 125.

7 Hypophysectomized for 28 days, no. 265, exhibiting flattened epithelium, and large lumina. Bouin-Delafield-eosin stained. × 125.

8 Hypophysectomized for 39 days, no injections, no. 143. Champy, unstained. × 537.

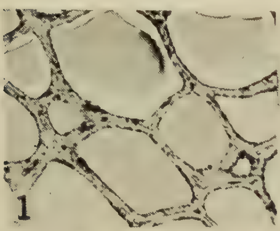
9 Activated 6 days with anterior pituitary, centrifuged 60 minutes, no. 328, showing stratification of the nuclear material. Bouin, Delafield-eosin stained. × 537.

10 Hypophysectomized 53 days, injected 21 days with anterior pituitary, no. 181, illustrating folding of epithelium almost obliterating the lumina. Champy, unstained. × 125.

11 Hypophysectomized 59 days, injected 15 days, no. 174, indicating an increase in numbers of Langendorff cells (degenerating chief cells). Champy, unstained. × 537.

12 Activated 6 days with anterior pituitary, centrifuged 60 minutes, no. 329, with the Golgi bodies displaced centripetally. Champy, unstained. × 125.

13 Outlined follicle of figure 12. Oil immersion. × 1212.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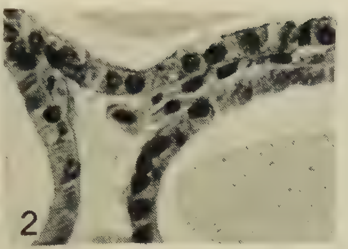
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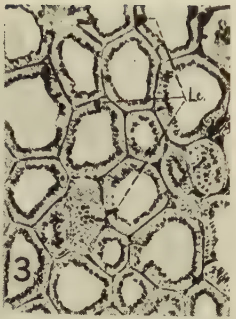
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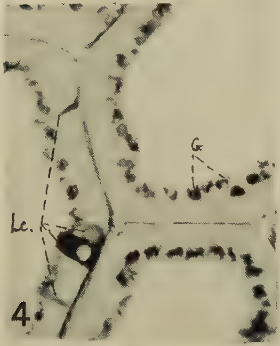
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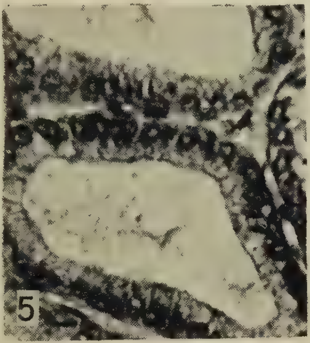
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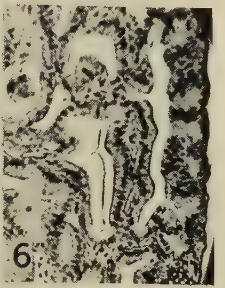
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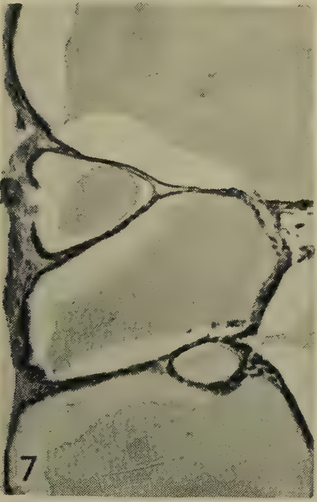
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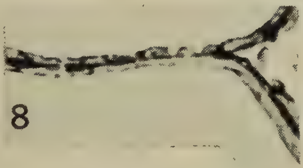
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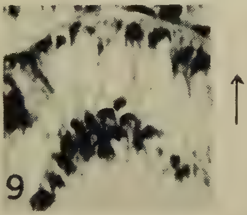
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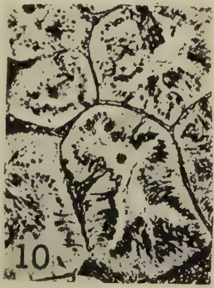
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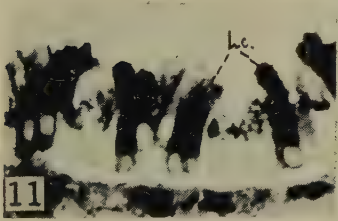
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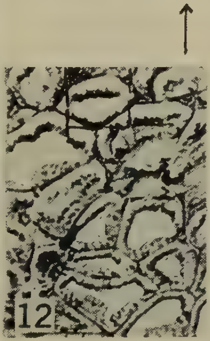
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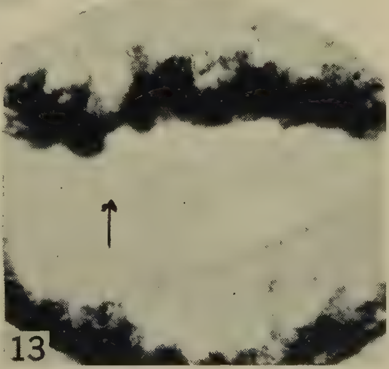
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PLATE 2

EXPLANATION OF FIGURES

All figures are × 3500 and are based on a study of slides using different fixatives and stains. In all figures the base of the cell is toward the bottom of the page except in figure 28. In centrifuged material, arrows indicate the direction of the centrifugal force.

14 Epithelial cell, normal control, no. 42.

15 Polar view of Golgi apparatus of normal, no. 42.

16 Cell after 6 days of anterior pituitary activation, no. 332. Golgi study.

17 Same as figure 16, but stained for nuclear study.

18 Activated 6 days, centrifuged 60 minutes. Nuclear displacement.

19 Polar view of normal cell. Nuclear appearance.

20 Nuclear stratification after 6-day activation and centrifuging 60 minutes.

21 Activated 6 days, centrifuged 20 minutes. Golgi apparatus displaced.

22 Activated 6 days, centrifuged 60 minutes. Golgi apparatus displaced.

23 Langendorff cell of normal thyroid.

24, 25, and 26 Hypophysectomized 30 days. Golgi apparatus apical, to one side, and basal respectively.

27 Circular and crescent-shaped Golgi apparatus in polar view of figures 24, 25 and 26.

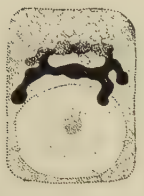
28 Shape of centrifuged nucleus when forced against the base of the cell.

29 Langendorff cells activated 6 days.

30 and 31 Further stages of degeneration of Langendorff cells.

32 Hypophysectomized 28 days. Edge view of chief cell.

33 Hypophysectomized 28 days. Surface view of chief cell.



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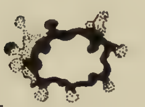

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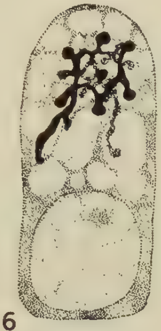

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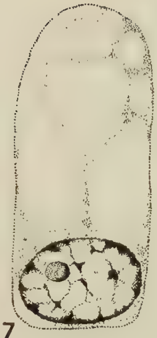

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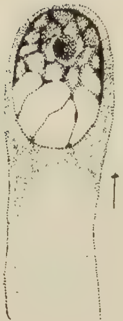

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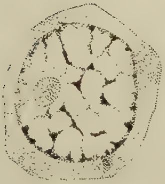

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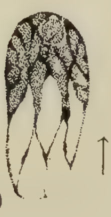

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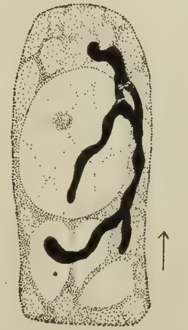

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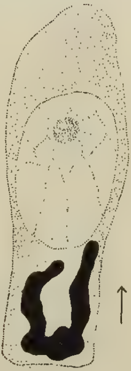

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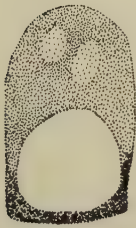

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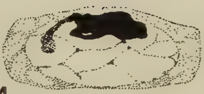

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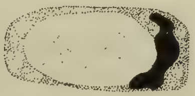

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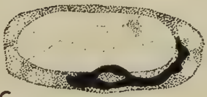

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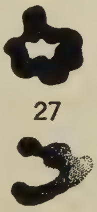

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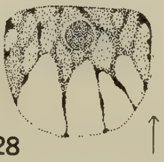

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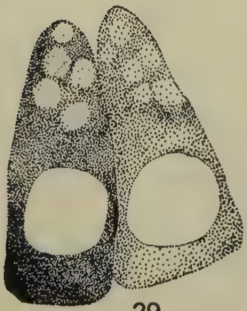

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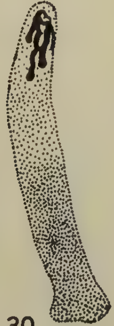

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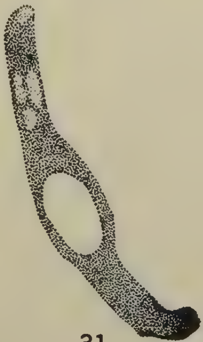

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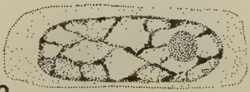

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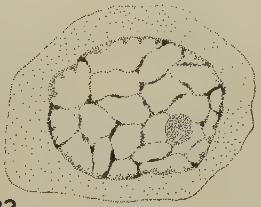

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METAMORPHOSIS OF THE PUBIC SYMPHYSIS

II. THE GUINEA PIG

ELBERT B. RUTH
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TWO PLATES (SEVEN FIGURES)

The pubic symphysis of the guinea pig offers an interesting study in sexual dimorphism, as has been noted many times, because of the spectacular relaxation of all the pelvic joints and the formation of a wide ligament between the symphyseal faces of the pubic and ischial bones in the female. This relaxation is presumably related to the relatively large size of the fetuses. The experimental work of Hisaw ('29) has called attention to the general trends of development which bring about the dimorphism and make relaxation possible.

Examination of the male symphysis shows it to be a primary synchondrosis. In this type of joint the interosseous medium is at first hyaline cartilage. This is gradually replaced by bone, as skeletal development progresses. The final picture of such a joint shows almost complete replacement of the cartilaginous interosseous medium, with a fusion of the bones concerned. The female symphysis, on the other hand, while developing early as a primary synchondrosis, begins to differentiate, before birth, an incipient dimorphism that reveals the sex of the animal without the examination of any other parts. In both the male and female, the ischial as well as the pubic rami are involved in the formation of the symphysis.

The first indication of skeletal tissues in the pelvic region of the guinea pig is a condensation of the scleroblastema subsequent to the eighteenth day of gestation. By the twenty-first

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day the skeletogenous areas are marked out by prochondral zones. As these become more extensive, hyaline cartilage is differentiated, outlining the position of the definitive pelvis. By the thirty-fourth day the now well-differentiated pubic and ischial cartilaginous elements of the symphysis have come into close approximation, and have fused apparently—the line of fusion being marked by the closely packed and very numerous cells. At this time an ossification center has appeared in the ilium. The forty-seventh day of gestation shows ossification centers in the pubic and ischial elements of the innominate cartilages, and the cartilaginous arch is much stronger at the symphysis. The line of fusion between the ischial and pubic elements of each side is now much less apparent (fig. 6).

From this stage of development, on through postnatal life to maturity, the development of the male symphysis consists chiefly of a progressive ossification of the pubic and ischial rami of each innominate bone, resulting in the gradual replacement of the symphyseal hyaline cartilage by bone. At 1 day of age, the hyaline cartilage forms a wide, uninterrupted plate between the osseous elements (fig. 1). On the right side of the figure the approach of the ossification process is seen laterally, in the large, vesicular lacunae and shrivelled cells; and more medially, in the flattening of the cells and the beginning of their arrangement in columns.

The replacement of the symphyseal cartilage by bone, and the resultant narrowing of the interischiopubic plate takes place gradually until the third week of postnatal life. By the end of the third week, however, the progress of ossification toward the mid-line has slowed to such a degree that proliferation of the cartilage occurs at about the same rate as the ossification (fig. 2). The shift in the relative rate of development of cartilage and bone permits the pelvic canal to grow in diameter—a fact readily observed even without actual measurement. This developmental relationship between the cartilage and bone remains approximately the same until maturity, when the rate of growth of the bone begins to exceed that of the cartilage. In this manner the cartilage is gradually replaced by bone, much like the process seen in the union of an epiphysis with the diaphysis of a long bone. By the middle of the second year most of the cartilage has been removed or replaced by loose irregularly arranged connective tissue (fig. 3). At this time some osseous trabeculae pass completely across the symphysis, while hyaline cartilage is found at the extreme anterior and posterior ends. Many osteoblasts are found lining the symphyseal faces of the bones where all of the cartilage has been removed. While this was the oldest stage studied, it seems quite probable that the osseous union becomes considerably stronger as age advances.

In contrast with the development of the male symphysis already described, the embryonic female symphysis (while remaining essentially as a continuous bar of hyaline cartilage) shows the formation of an intracartilaginous fissure by the fifty-sixth day of gestation (fig. 4). The fissure forms in the inter-pubic region of the symphysis by a cataplastic metamorphosis of the hyaline cartilage. The cartilage cells become elongated and the hyaline matrix disappears, leaving the cells free. These cells take on the nature of fibroblasts, and give rise to scattered fibers of loose connective tissue. As this process becomes more extensive, the cartilage bordering the fissure gradually becomes fibrous. This newly formed fibrocartilage, in turn, is continuous with a relatively dense fibrous tissue which exists in some parts of the fissure. In other places the fissure contains very loose fibrous connective tissue, while some areas are wholly without fibrous tissue. At the age of 3 weeks the pubic cartilages are completely separated, but the symphyseal cartilage is still continuous in the ischial region. As development proceeds, the fissure becomes longer, and extends into the area between the ischial rami. At 5½ weeks the fissure is complete as seen in some of the microscopic sections, and incomplete in others, being bridged across by hyaline cartilage in these interrupted areas. These cartilaginous bridges are in no fixed position, but vary somewhat in different animals. The mature nulliparous female shows little change from this state (fig. 5), although ossification is progressing slowly. The cartilage cells bordering the advancing ossification process are arranged in cell nests, instead of the usual column formation. It is interesting to note that this same arrangement of cartilage cells at the symphysis is seen in mature males, seemingly becoming more apparent with the slowing up of ossification. On the other hand, the younger males show the usual column formation of cells, as do the young females up to 10 days old. This phenomenon suggests that the rate of ossification may influence the arrangement of the cartilage cells that precede the ossification process.

As the mature female grows older, the symphyseal cartilage which remains covering the symphyseal face of the ischium and pubis is reduced, gradually, until it no longer exists. At this time all cartilaginous connection between the two innominate bones is lost. With the removal of the cartilage, there occurs a proliferation of the symphyseal fibrous connective tissue, which becomes progressively denser and forms a narrow ligament between the bones. The fiber bundles lie parallel with the long axis of the symphysis, gradually bending laterally at the anterior and posterior extremities of the osseous elements, to become continuous with the transversely arranged bundles of fibers of the anterior and posterior (arcuate) ligament of the joint. The symphysis at this time resembles a syndesmosis (fig. 7).

DISCUSSION

It must be noted that in the adult female guinea pig there are two types of symphysis: 1) The chondro-fibrous type seen in young adults, in which the symphyseal faces of the pubic and ischial rami are covered with hyaline cartilage (which shows a medial gradation through fibro-cartilage to a more or less dense fibrous tissue) with partial or complete separation of the two innominate bones; and 2) the osseo-fibrous type found in older adults, in which the hyaline cartilage has disappeared from the symphyseal faces of pubic and ischial rami, and the bones are connected by a narrow symphyseal ligament of dense fibrous connective tissue.

The detailed observations of the developmental changes in the symphysis indicate the course of events which make possible the rapid formation of the wide ligament found in the parturient female, by a hyperplasia of the fibrous structure already present. Hisaw ('29) observed that the ovarian hormones control this sexual dimorphism, and demonstrated experimentally that early castration of the young female results in the formation of the male type of pelvis, which cannot be altered by the action of ovarian hormones administered subsequently. On the other hand, it is possible, by feminizing young males with ovarian grafts after early castration, to effect some degree of relaxation. After the pelvis has reached its adult condition it is apparently impossible to modify it.

Two of the adult virgin female symphyses (in which the cartilage covering the symphyseal faces of the bones has disappeared) have about the appearance of the symphysis of the multiparous female, with two exceptions: 1) The ligament is narrower, and 2) shows two distinct zones. The zone immediately adjacent to the bone is made up of a great number of fibroblasts, with much fewer fibers; while the central zone (which is widest) is made up predominantly of collagenous fiber bundles—the cells being relatively scarce. The exact significance of this picture is not understood. It is possible that the activity of these fibroblasts in the elaboration of fibrous tissue, may show some periodicity in relation to the periodic relaxation noted by Pommerenke ('33) in the symphysis of virgin females during oestrum. It may be that this is only a phase of the process by which the definitive adult ligament is formed.

SUMMARY

The male symphysis, as its ossification begins, passes from the embryonic indifferent stage in which it is a continuous bar of hyaline cartilage, into an osseo-chondral phase. This phase first shows a period of rapid ossification, until the third or fourth week of postnatal life, after which there is a period of slow ossification, during which time the proliferation of the symphyseal cartilage keeps pace with the ossification process, and the pelvic canal grows in diameter until it reaches the adult size. The final stage of development is seen in older animals with the removal of the symphyseal cartilage and the partial or complete union of the osseous elements.

The female symphysis passes from the embryonic indifferent stage into the chondro-fibrous stage, wherein the sexual dimorphism first appears by the formation of a fissure containing a few fibroblasts and scattered fibers, which give rise to a more or less complete separation between the skeletal elements of symphysis, which are held together by a narrow ligament. As the cartilage disappears from the symphyseal faces of the bones, the osseo-fibrous stage (which is the definitive condition of the female symphysis) begins, and does not change significantly throughout life. In this stage the symphyseal ligament is altered somewhat in width by succeeding pregnancies, and after the first pregnancy never regains its virginal condition, although it does contract considerably between pregnancies.

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PLATES

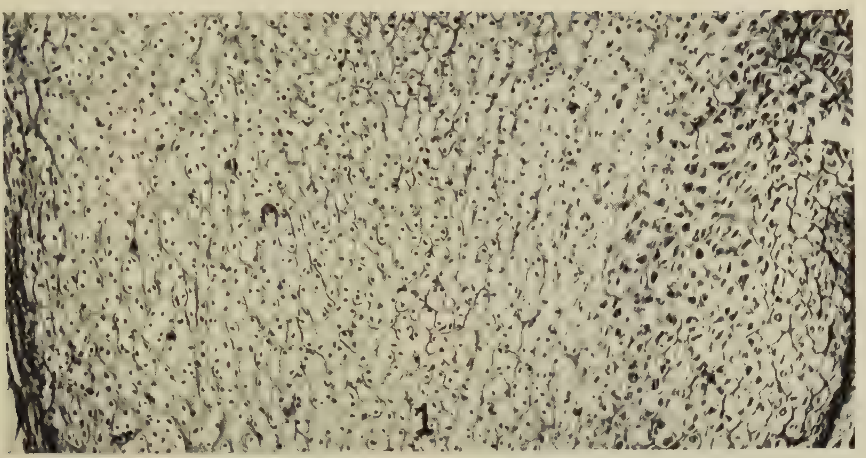
PLATE 1

EXPLANATION OF FIGURES

1 Coronal section through the symphysis of a male guinea pig 1 day old. × 100.

2 Coronal section through the symphysis of a male guinea pig 3 weeks old. × 100.

3 Coronal section through the symphysis of a male guinea pig 1½ years old. × 100.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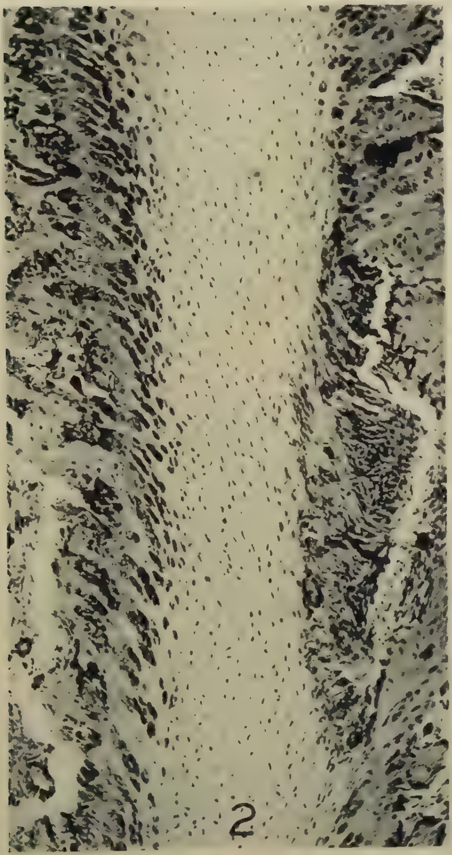
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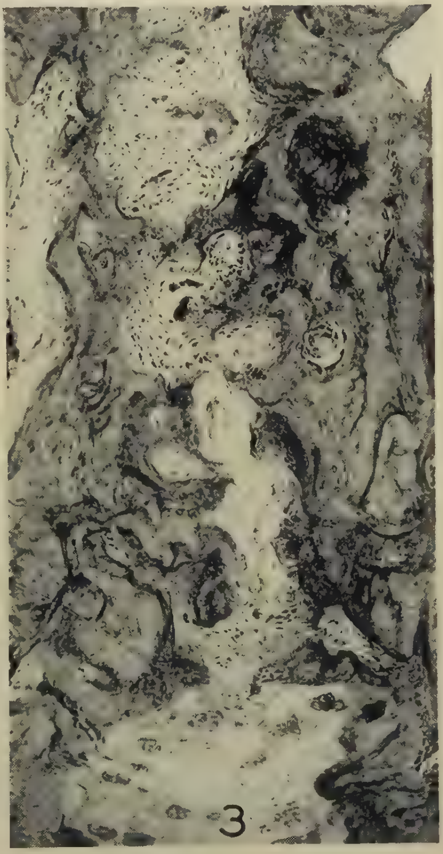
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PLATE 2

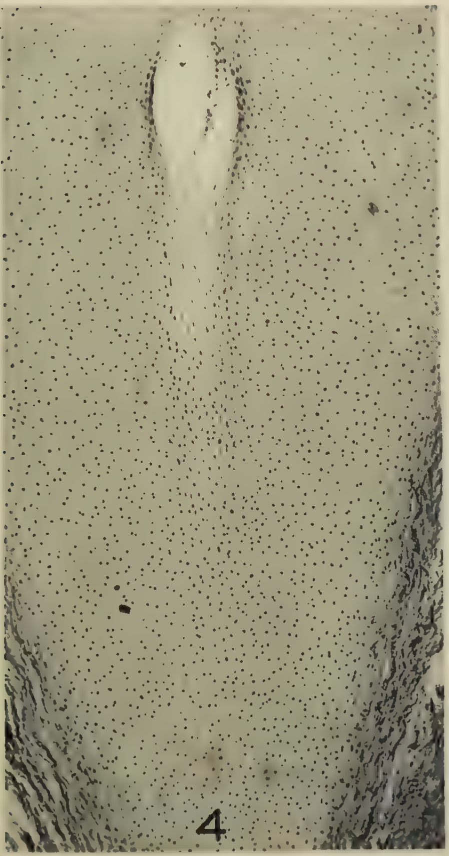
EXPLANATION OF FIGURES

4 Coronal section through the symphysis of a female guinea pig fetus of 56 days' gestation, showing the interpubic fissure. × 100,

5 Coronal section through the symphysis of a young adult virgin guinea pig, showing the fissure interrupted by a bridge of cartilage. × 60.

6 Coronal section through the symphyseal region of a female guinea pig fetus of 47 days' gestation, showing the indifferent condition common to both males and females. × 100.

7 Coronal section through the symphysis of an older adult multiparous guinea pig, showing the osseo-fibrous condition. × 25.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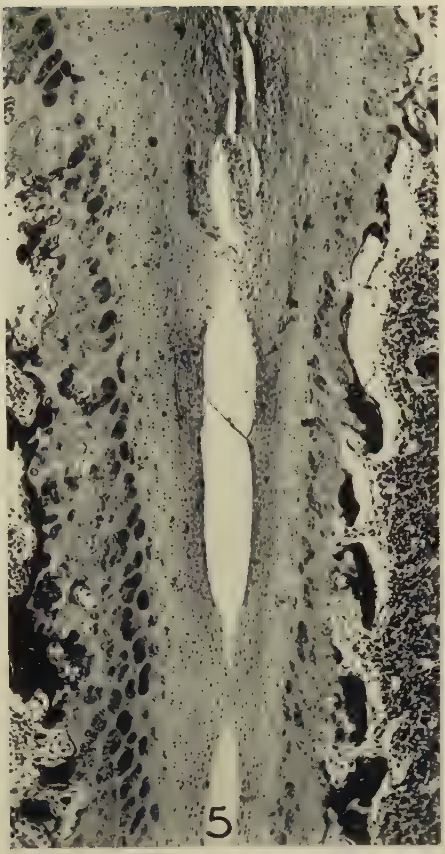
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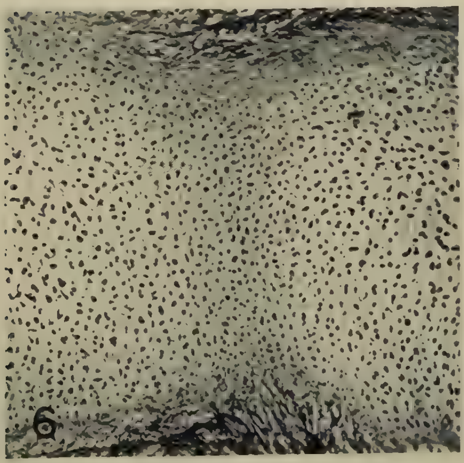
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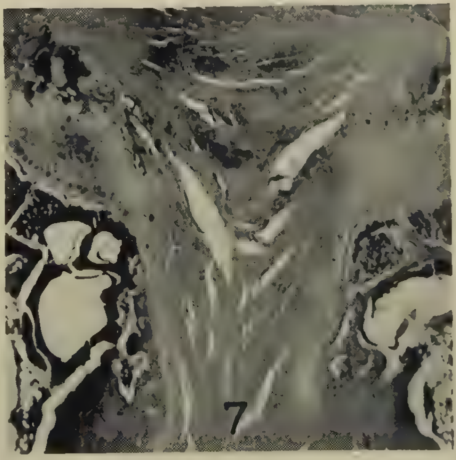
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THE CALCIUM DEPOSITS IN NERVE CELLS OF WHITE RAT AFTER INJECTIONS OF UREA AND CHOLESTEROL

ROSS C. MacCARDLE

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ONE PLATE (SIX FIGURES)

During the course of some studies on the effect of temperature, excess cholesterol and urea on the lipoids, water content and cytoplasmic structures in nerve and liver cells, the writer became interested in the unusual deposition of calcium which appeared in silver preparations of nerve cells, especially after treatment with excess cholesterol or with urea. Although no gross chemical analysis has been made of these tissues, it was thought that a public report of this unusual phenomenon, might result in some correlation of the already conflicting data on the problem of calcification.

Calcification of nerve cells is not of frequent occurrence, although deposits of calcium often accumulate around ganglia and nerve fibers. Degenerative ganglion cells of the brain often become calcified (Wells, '25). Rukstinat ('35) has reported an interesting case of focal calcification of the brain of an idiot child. The pineal gland often contains calcified cysts in the neuroglial cells (Hortega, '29). Such calcifications have been generally attributed to acute infections, to chronic disease such as epilepsy, and to intoxications such as carbon monoxide poisoning.

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MATERIAL AND METHODS

Eighty white rats (of The Wistar Institute strain), kept on a normal diet, were divided into two groups; 1) forty of them received daily injections intraperitoneally of 4 cc. of a saturated solution of cholesterin (Merck) in olive oil; and 2) forty received daily injections of 5 cc. of a 3% aqueous solution of urea (Merck). Proper normal controls were kept. Specimens were killed at intervals of 5 days, over a period of 42 days. The brain and cord were studied cytologically for calcium deposits, using the silver nitrate technique of Van Kossa. Sudan III and osmic acid were used to identify fats.

CHOLESTERIN EXPERIMENTS

After 5 or 6 days, animals receiving cholesterin injections usually become weak and later partially or completely paralyzed in some of their organs, such as feet, legs and neck.

In all but two animals, large intracellular deposits of calcium (phosphate?) may be seen in the ganglion cells of the medulla oblongata, and in the motor cells of the spinal cord (compare figs. 1, 2, 3, 4). This deposition apparently begins after 18 days or more of injections. In many instances, large clumps of crystals of free cholesterin appear (fig. 5). Fatty globules begin to accumulate at about the twentieth day. They appear in large vacuoles in the cytoplasm and in the intercellular spaces.

UREA EXPERIMENTS

These rats seem to possess a greater resistance to excess urea than to cholesterin, for the signs of degeneration did not begin to appear until after about 15 or 20 days, and calcium deposits did not appear until after 30 days of treatment (fig. 6). Most of them died after the thirty-fifth day. Fatty deposits were evident generally after 30 days of treatment. In some hypertrophied cells of the medulla, masses of certain crystals appear, which I interpret as being urea crystals; although I cannot conceive of their being insoluble in the water of the cytoplasm.

DISCUSSION

Little is known of the mechanism of pathological calcification of the soft tissues of the body. Several interesting theories have been proposed concerning it. Hofmeister ('10) suggested that deposition of calcium in arteries might be due to decreased carbon dioxide of the blood with resulting increase in alkalinity, thus favoring the precipitation of calcium salts. Klotz ('05) believed that fatty degeneration and formation of calcium soaps, which subsequently form calcium phosphate and calcium carbonate, constitutes the mechanism of calcification. But Wells ('25) points out that "when calcium soaps are implanted in the tissues, they do not become transformed into calcium phosphate and calcium carbonate, but are largely absorbed and removed." Robison ('23) records evidence of the presence in certain ossifying tissues of an enzyme, phosphatase, which liberates phosphoric acid, binding in turn the calcium. Hueper ('27) recognized the importance of vitamin D in normal ossification, and Ham ('32) states that "enormous single doses of irradiated ergosterol will produce massive calcifications" in blood vessels. Excellent bibliographies on this subject appear in the masterly reviews of Barr ('32) and of Wells ('33).

Calcium deposits have also been observed in electronegative injured portions of frog's muscle (Burge, et al., '35), and in Elodea cells (Mazia, '36).

The calcium deposits after cholesterin treatment in rats, may be explained easily on the basis of Klotz' theory of the liberation of fatty acids, the formation of calcium soaps, and the subsequent transformation into calcium phosphate. Large deposits of fatty material appear in cells undergoing calcification. However, calcium may appear in kidney cells (to be reported later) without previous accumulation of fat. In the absence of chemical and physico-chemical analyses of the fluids and tissues of these animals, it is difficult to draw any real conclusion.

In the case of calcium deposits after treatment with urea, it is still more difficult to understand the relationship between the urea, fat and calcium. It is, indeed, conceivable that urea can stimulate the formation of phosphatase, and certain that it may provide a high degree of alkalinity so that calcium can then be precipitated. Hofmeister pointed out that during the active stages of urine secretion, the kidney tissue is highly alkaline, whereas during rest it is highly acid. Thus the nervous tissue might become highly alkaline during the elimination of excessive wastes, and so precipitate calcium. And certainly the cell would possess an abundant supply of waste in these animals, since urea is a very rapidly penetrating substance.

SUMMARY AND CONCLUSIONS

1. Deposition of calcium in ganglion cells of the medulla oblongata and in the motor cells of the spinal cord, of the white rat, was induced by intraperitoneal injections of cholesterin and urea separately.
2. Crystals of cholesterin, and sometimes crystals of urea (?) in giant cells, were deposited in cytoplasmic vacuoles.
3. Although fatty accumulations generally preceded the deposition of calcium, no evidence of the formation of calcium soaps could be detected.
4. It is believed that urea stimulates a higher degree of alkalinity, thus favoring the precipitation of calcium.

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PLATE 1

EXPLANATION OF FIGURES

All figures were drawn with the aid of an Abbe camera lucida under a magnification of × 1500. All figures are taken from preprations made according to the Von Kossa silver nitrate technique.

1 Ganglion cell from medulla oblongata of normal rat.

2 Motor cell from ventral horn of spinal cord. Normal.

3 Ganglion cell from medulla of cholesterin treated rat. Note intra- and intercellular deposits.

4 Motor cell from spinal cord of cholesterin treated rat.

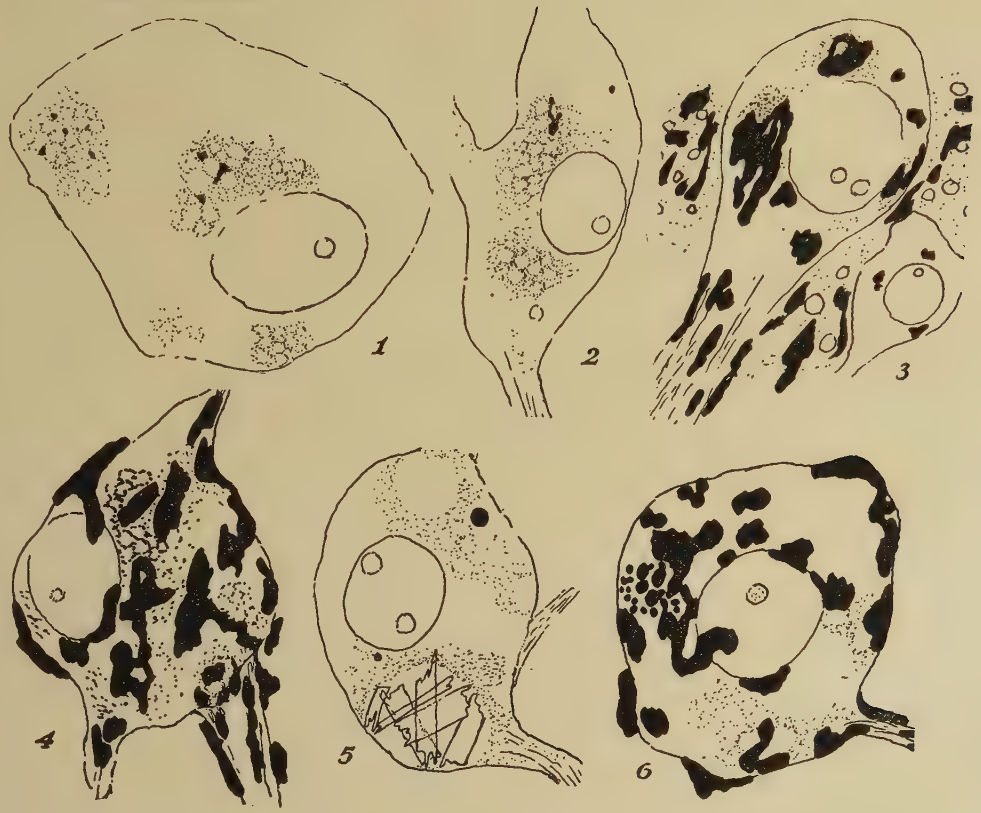
5 Motor cell from spinal cord of cholesterin treated rat. Fresh. Animal was emaciated and hind legs and back muscles paralyzed.

6 Ganglion cell from medulla of urea treated rat.

CALCIUM DEPOSITS IN NERVE CELLS

ROSS C. MacCARDLE

PLATE 1



THE PERITONEAL STOMATA

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ONE TEXT FIGURE AND TWO PLATES (FOUR FIGURES)

INTRODUCTION

At the present time, despite much investigation, the peritoneal surface of the diaphragm remains something of a histological puzzle. It has long been recognized that particulate matter, injected into the peritoneal cavity, will pass rapidly through the peritoneum and into the extensive sub-diaphragmatic lymphatic plexus, yet neither the anatomy of the structures in question nor the mechanism of the process of absorption is thoroughly understood.

Peritoneal stomata through which von Recklinghausen (1863) claimed to have observed the passage of particles, or smaller stomata with which the names of Cohnheim and Arnold are associated, are anatomically adequate to account for rapid absorption of relatively large particles. The observation of von Recklinghausen is now generally discredited and since the work of Hertzler ('01) any intercellular openings in the peritoneum, demonstrable by silver impregnation, have been regarded as artefacts. It should be pointed out, however, that the occurrence of peritoneal stomata has never been disproven. They have been abandoned on the grounds that they were probably artefacts and replaced by other less convincing concepts. We may summarize the different ideas on diaphragmatic absorption as follows: 1) Particles are carried into the subdiaphragmatic lymphatic plexus by free phagocytes. 2) Particles are phagocytized by the peritoneal mesothelium and transferred in some way to the lymphatic lumen. 3) Particles are forced between the cells of the peritoneal mesothelium by respiratory movements, intra-abdominal pressure, and the rubbing effect of viscera. Most investigators believe that more than one of these factors is operative. The literature on this subject has become quite voluminous and is reviewed in detail by Cunningham ('26).

This investigation consists of a histological study of those areas of diaphragmatic peritoneum concerned in the absorption of particulate matter. In the latter part of this work the anatomical findings are correlated with the mechanism of the absorptive process.

MATERIALS AND METHODS

It was desired to select for intraperitoneal injection some large particle whose position within the peritoneum during absorption could be definitely determined. Frog erythrocytes were selected for this purpose. Smaller particles such as chick and mouse erythrocytes, India ink and 1% carmine suspension were also used in confirming certain points.

The white mouse was used exclusively as the experimental animal since its small thin diaphragm lends itself readily to the preparation of whole mounts.

As a routine procedure 1 cc. of particulate matter was injected intraperitoneally and after an interval of 5 to 30 minutes the animal was killed by decapitation, the abdomen inflated with Bouin's fluid or 10% formalin and the diaphragm fixed in situ. After the diaphragm was removed, by carefully cutting around its periphery, it was stained with hematoxylin and eosin, dehydrated, cleared in xylol and mounted with the peritoneal surface uppermost in damar. Diaphragms were prepared for serial sectioning according to the double embedding technique of Jones ('36). This method avoids any fragmentation of the delicate structures in question here.

The following technique yielded the most instructive preparations for our purposes: The abdomen was inflated with silver nitrate 1:300 solution. After 2 minutes this was withdrawn with a syringe and replaced with a 10% formalin solution. After fixation the diaphragm was removed, stained with hematoxylin and eosin, dehydrated, cleared in xylol and mounted with the peritoneal surface uppermost. By this method the intercellular cement was impregnated with silver and the nuclei and cytoplasm of peritoneal mesothelium, lymphatic endothelium, and frog erythrocytes were likewise stained.

Other diaphragms were prepared as whole mounts following a 10-minute impregnation in situ with a 1:300 silver nitrate solution.

OBSERVATIONS AND RESULTS

The subdiaphragmatic lymphatic plexus. The vessels which constitute this plexus lie immediately subjacent to the peritoneum and radiate from the central tendon, occupying the depressions between muscle fascicles. Toward the periphery of the diaphragm these vessels drain into small efferent channels which pass through the musculature of the diaphragm and communicate with a small plexus on its pleural surface.

This plexus is described for the dog by MacCallum ('03) who speaks of the large subperitoneal lymphatic spaces as the lymphatic 'lacunae' and the triple layered membrane which separates a lymphatic lumen from the peritoneal cavity as the 'roof' of a lymphatic lacuna. These 'roofs' are composed of peritoneal mesothelium resting upon a fenestrated basement membrane and, in contact with the basement membrane, the endothelium of a lymphatic lacuna. It is with these triple layered 'roofs' that we are primarily concerned here since it is through them that particles and solutions must pass during lymphatic absorption.

Lymphatic lacunae of the mouse. The relation of a subdiaphragmatic lymphatic lacuna to the overlying peritoneum is shown in figure 2. This is a stereogram made from a camera lucida sketch of a whole mount of the diaphragm which was impregnated with silver and stained with hematoxylin and eosin. By focusing upon different levels, the deeper structures were drawn in their proper relation to the peritoneum. A portion of the peritoneal mesothelium and basement membrane of the roof is cut away in order to show the underlying lymphatic endothelium. In this figure the 'roof' is shown to bulge above the general level of the diaphragmatic peritoneum. This effect is due to fixation for in the fresh diaphragm the 'roofs' lie below the general level of the peritoneum and the volume of the lacunae is correspondingly decreased. As will be shown later this fact is of extreme importance in regard to the mechanism of absorption.

The cells of the peritoneal mesothelium of the roofs differ from those of the general peritoneum in that they are smaller and less flat with an accumulation of slightly basophilic cytoplasm in the region of the small dark nuclei. The lymphatic endothelium of the 'roof' is composed of rows of more or less oblong plates, the long axes of which are situated at right angles to the length of the muscle fascicles of the diaphragm. The nuclei of these cells are arranged in parallel rows and the cytoplasm is accumulated in the region of the nuclei. The margins of these cells are thin and wavy in contrast to the heavy serrated borders of the endothelium of the floor of the lacunae.

Peritoneal stomata. In preparations of the diaphragm impregnated with silver may be seen the curious rings between cells of the peritoneum. These rings, regarded by many as artifacts due to stretching, drying, precipitation of silver chloride by the serous fluid, etc., were always quite numerous in our preparations regardless of how carelessly or carefully the diaphragm was handled. It is a very significant fact that rings or stomata always occur in the 'roofs' but never in the general peritoneum of the diaphragm, body wall, etc.

Whole mounts of the diaphragm impregnated with silver and stained with hematoxylin and eosin give the most instructive picture of the stomata. With this method the intercellular lines are very thin and peritoneal stomata appear not as rings but as oval openings within the accumulations of intercellular cement at the junction of usually three cells. These openings represent the peritoneal ends of short canals (fenestrae) which passes through the basement membrane to abut against the intercellular cement of the lymphatic endothelium (fig. 1 a and b). These fenestrations of the basement membrane will be considered more fully in connection with the absorption of particles. Openings comparable to the peritoneal stomata do not occur in the endothelium of the 'roof.' However, accumulations of intercellular cement do occur and occasionally in focusing downward through the larger of the peritoneal stomata there may be seen small oval openings between endothelial cells which seem to have arisen from a rarefaction of the intercellular cement. It is apparent from these observations that if particles pass through the peritoneal stomata they must pass also between cells of the lymphatic endothelium.

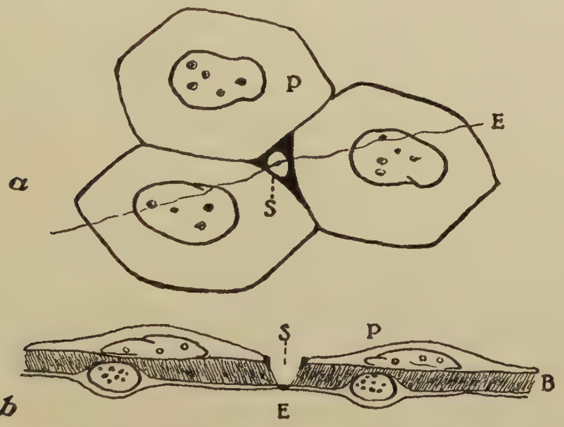

Fig. 1 a, surface view illustrating relation of peritoneal stomata to intercellular cement of lymphatic endothelium. b, section through a peritoneal stomata of the diaphragm. Legends: S, peritoneal stomata; B, fenestrated basement membrane; P, peritoneal mesothelium; E, intercellular cement of lymphatic endothelium of 'roof.'

Absorption of frog erythrocytes and India ink. Observations on the absorption of these particles demonstrate the significance of peritoneal stomata. Within 5 to 30 minutes after peritoneal injection the 'roofs' of the lymphatic lacunae are studded with frog erythrocytes which have become caught in passing through the stomata (fig. 3). Preparations impregnated with silver and stained with hematoxylin and eosin show erythrocytes within the peritoneal stomata and deep to these, between cells of the lymphatic endothelium. In one preparation consisting of one-half of a mouse diaphragm we have counted over 600 frog erythrocytes. These cells are so situated within the 'roofs' that one end of each erythrocyte lies within the peritoneal cavity and the other projects into the lymphatic lumen (fig. 4). Erythrocytes are constricted in passing through the stomata, in some instances to such an extent as to be just visible. The process here is analogous to the diapedesis of erythrocytes in the inflamed mesentery of the frog. The portion of the erythrocytes which has penetrated the stomata is frequently hemolyzed and the nuclei sometimes extruded (fig. 4, cells 1 and 2). The lymphatic lacunae and their efferent channels contain many erythrocytes, some vacuolated and hemolyzed with pycnotic or extruded nuclei, others apparently undamaged by the absorptive process. These observations give us some idea of the size of the stomata. From the degree of constriction of the frog erythrocytes it is apparent that many of the stomata are from 2 to 6 μ in diameter (see cell 3, fig. 4). Other stomata are certainly much larger since many erythrocytes are found undamaged in the lacunae.

Studies were made also of the absorption of chick and mouse blood. These erythrocytes were found in large numbers in the lacunae but only in a single instance was a chick cell found within one of the smaller stomata and never a mouse erythrocyte. Apparently the stomata are large enough to easily accommodate these smaller particles.

Within a few minutes after intraperitoneal injection the diaphragmatic peritoneum is diffusely coated with clumped particles of India ink. In many situations these particles outline the peritoneal cells. Upon exposing the diaphragm to a stream of water for several seconds all the ink washes off except the clumps of ink within the fenestrae[1] of the basement membrane (fig. 5) which appear as black canals or plugs leading down to the lymphatic endothelium. This procedure demonstrates that the fenestrations of the basement membrane are essential parts of the stomata. Fine ink particles are evenly distributed over the diaphragm, adherent to the peritoneal cells and not within their cytoplasm.

DISCUSSION

The results described here confirm the opinion of the earlier writers that particulate matter is absorbed at certain definite points on the peritoneum which they demonstrated by silver impregnation and called stomata. None of our observations support the view of MacCallum ('03) or Florey ('27) that the absorption of particles is a general intercellular process. This view is based upon the observation that India ink particles appear on the peritoneum in a pattern quite similar to that shown after the application of silver nitrate. We found that this picture is due to the settling of particles in the depressed areas between cells from which it can readily be washed off except in the regions of the stomata. Nor is it possible to correlate such a theory with the findings on the fenestrations of the basement membrane (Bizzozero and Salvioli, 1876; Muscatello, 1895). It is interesting to note in this connection that Hertzler ('01) who denied the natural occurrence of both the peritoneal stomata and the fenestrations of the basement membrane used the peritoneum of the abdominal wall for his studies.

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[1] The basement membrane is described by Bizzozero and Salvioli (1876), Muscatello (1895), from strips of basement membrane torn off following maceration of the peritoneum in Müller's fluid and alcohol. They describe this membrane as being homogeneous and imperforate every where except over the lymphatics of the diaphragm where fenestrations occur. Muscatello determined these fenestrations to be from 4 to 16 μ in diameter in the dog.

In whole mounts of mouse diaphragm stained heavily with hematoxylin fenestrations may be observed. These occur only in the basement membrane of the 'roofs' of lymphatic lacunae and appear as clear oval areas about the size of mammalian erythrocytes.

Cunningham ('22 a and b) was of the opinion that particles pass through the cytoplasm of the peritoneal mesothelium and lymphatic endothelium, this absorption being a function of the vital activity of these cells and probably assisted in some way by pressure of viscera, etc. It is difficult to account for the fact that particles appear in the lymphatics within 3 to 5 minutes after intraperitoneal injection (Higgins and coworkers, '30), and that an animal may absorb one-fifth of its own blood volume in 5 hours (Siperstein and Sansby, '23), on a basis of phagocytosis by peritoneal mesothelium. We were never able to observe erythrocytes within cells of the peritoneal mesothelium. The more abundant cytoplasm of the roof cells does suggest some specialization in the direction of phagocytosis. In checking this point we found within 24 to 48 hours after intraperitoneal injection, a few granules of carmine in the peritoneal mesothelium of the 'roofs' and a perinuclear arrangement within the lymphatic endothelium of the 'roof.' It is concluded that the vital activity of the peritoneal mesothelium of the 'roofs' is greater than that of the general peritoneum but nevertheless plays an insignificant role in absorption.

The question now arises as to whether the stomata are fixed communications between the peritoneal cavity or whether they are open only at certain times. It is apparent that the stomata are actual openings at the time when absorption takes place, openings large enough to permit the absorption of frog erythrocytes. We define the stomata as openings in the peritoneum and basement membrane through which the peritoneal cavity comes into contact with the intercellular cement of the lymphatic endothelium (fig. 2, B). Now what is the mechanism by which these communications are completed and particles forced through into the lymphatic lumen?

It has been obvious for a long time that the movements of the diaphragm are concerned in the process of absorption. Florey ('27) determined a pressure of 2.5 cm. of water between the contracting diaphragm and the liver and believes the pressure sufficient to force particles into the lymphatics.

We have recently described experiments (Allen, '36) which indicate that particles are 'sucked' through the stretched peritoneum during the relaxation of the diaphragm. If a few drops of India ink are placed upon the peritoneal surface of the diaphragm and the diaphragm stretched once it may be observed after washing off the excess ink that the ink has passed through the 'roofs' and clearly outlines the lymphatic lacunae. Under the binocular microscope it is noted that the 'roofs' of the lymphatic lacunae are raised up out of the lumena by the stretching process in such a way as to increase the volume of the lacunae. This increase in volume gives rise to a relative negative pressure which is effective in 'sucking' suspensions and solutions through the stomata.

It is quite probable that this stretching of the diaphragm which is responsible for absorption is also effective in separating the lymphatic endothelial cells in the region of the stomata and thus completing the communications between the peritoneal cavity and the lymphatic endothelium. The fact that the roof cells of the lymphatic endothelium are situated with their long axes at right angles to muscle fascicles (fig. 1) supports this contention, since the greatest stretching occurs along the line of these fascicles.

The peritoneal surface of the diaphragm has been investigated so frequently in regard to the absorption of particles that some are likely to consider diaphragmatic absorption as a mechanism specialized for that purpose. Since particulate matter does not normally occur in the peritoneal cavity diaphragmatic absorption must be regarded as a mechanism for 'mopping up' the normally occuring serous fluid of the peritoneal cavity.

SUMMARY

The diaphragmatic peritoneum and subjacent lymphatic lacunae have been studied by a variety of methods and the lymphatic absorption of particles is described in detail.

Peritoneal stomata are normal and physiological openings in the peritoneum overlying lymphatic lacunae of the diaphragm. Through these openings fluid within the peritoneal cavity comes into contact with the intercellular cement of the lymphatic endothelium. Stomata are thus comparable to tissue spaces. As the diaphragm relaxes during expiration the endothelial cells are pulled apart in the regions of the stomata and particles and solutions are sucked through the stomata by a relative negative pressure in the lymphatic lumen. These findings correlate the anatomy of the structures in question with the mechanism of the process of absorption.

Some of the stomata were measured directly by the size of the particles within them and found to be from 2 to 6 μ in diameter. Evidence is presented which indicates that many of the stomata are much larger, possibly 10 to 15 μ in diameter.

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PLATE 1

EXPLANATION OF FIGURE

2 Stereogram of subdiaphragmatic lymphatic lacunae. (Compare fig. 1 a and b.)

THE PERITONEAL STOMATA
LANE ALLEN

PLATE 1

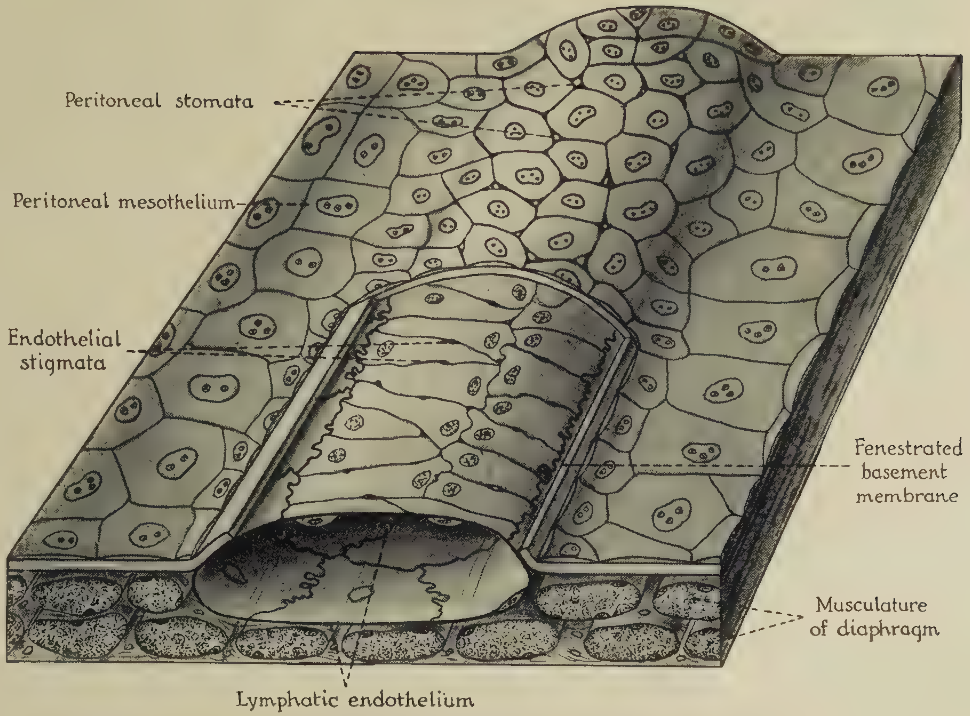

Peritoneal stomata
Peritoneal mesothelium
Endothelial stigmata
Fenestrated basement membrane
Musculature of diaphragm
Lymphatic endothelium

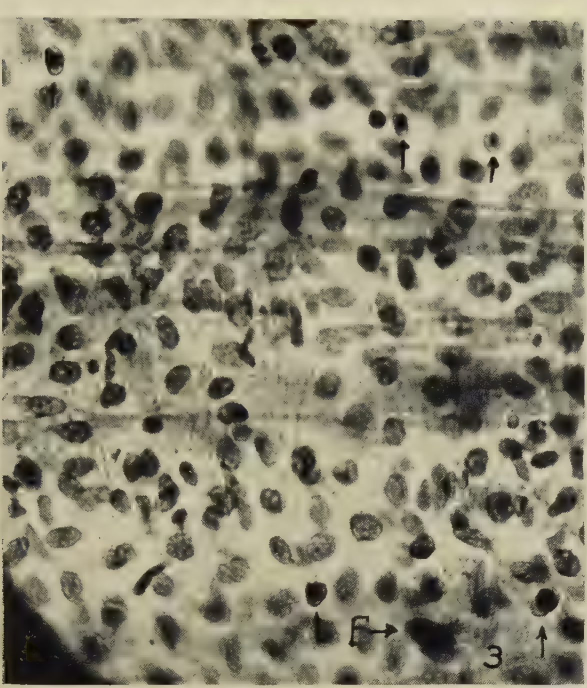
PLATE 2

EXPLANATION OF FIGURES

3 Absorption of frog erythrocytes. Arrows point to frog erythrocytes in peritoneal stomata. F, frog erythrocyte lying flat on peritoneum. Hemat., eosin. × 450.

4 Absorption of frog erythrocytes. Section of subdiaphragmatic lymphatic lacuna showing three erythrocytes passing through stomata of roof. Note partial hemolysis of absorbed portion of cell 1., extrusion of nucleus of cell 2 and diameter of stoma, cell 3. Hemat., eosin. × 800.

5 Demonstration of peritoneal stomata with India ink. × 250.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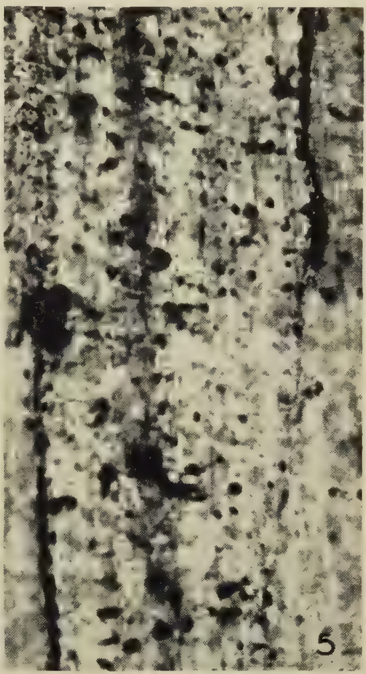
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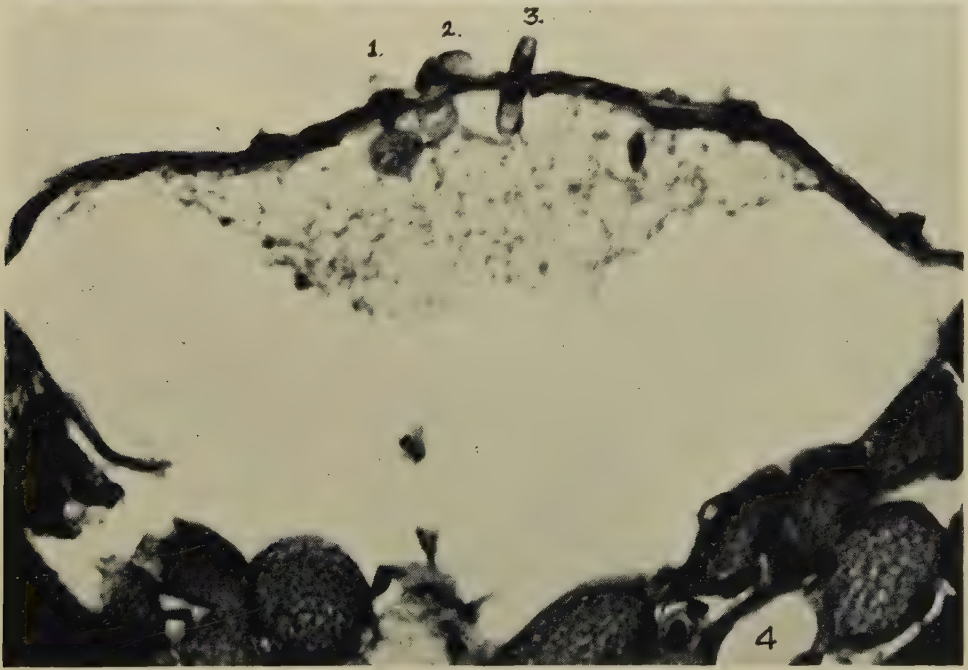
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NEUROFIBRILS IN LIVING GANGLION CELLS OF THE CHICK, CULTIVATED IN VITRO

PAUL WEISS AND HSI WANG
Department of Zoölogy, The University of Chicago

THREE FIGURES

There is still considerable dissension regarding the reality of neurofibrils. The arguments pro and contra have been reviewed comprehensively by Parker ('29) and Péterfi ('29). Whereas there seems to be no doubt that the fibrillar structures observed in fixed and stained preparations have some counterpart in the living cell, there is no agreement as to whether fibrillae exist in the living cell as discrete filaments, or whether they are merely present potentially in the form of oriented, ultramicroscopic elements which, in the process of fixation, coagulate into fibrillae.

In but a few instances, the actual existence of fibrillae in the living nerve cell and fiber has been proved directly by observation of the living object. Bozler ('27) described them in medusae; de Rényi ('29 b) and Kedrowski ('35) in crustacea. Speidel ('35) reported the appearance of a longitudinal striation in damaged and degenerating nerve fibers of amphibian larvae. Attempts, however, to detect fibrils in the undamaged nerves of vertebrates, have failed consistently (de Rényi, '29 a). Moreover, the method of teasing nerve fibers from their normal surroundings and transferring them into an artificial medium for immediate observation may vitiate the results. By manipulation, fibrils, if present, may be rendered invisible, and conversely, the presence of fibrils may be simulated by coagulation effects. These dangers are considerably lessened if nerve fibers are kept in prolonged cultivation outside of the organism. As a matter of fact, many workers in the field of tissue culture of nerves have been aware of the possibility that the existence or non-existence of fibrillae might be demonstrated most advantageously by this method.

One of the most experienced workers in the field, G. Levi, has reviewed the subject in a recent article ('34). His observations on the living and growing nerve fiber in tissue culture, supplemented by the observations of Mossa ('27) in dark field illumination, can be summarized in the statement that both the soma of the nerve cell and the axis cylinder are filled with a highly refringent substance, the elements of which appear to be oriented parallel to the axis of the fiber so as to make the interior of the fiber display a faint longitudinal striation. But no evidence for the existence of individual and discrete fibrillae has been forthcoming. Levi concludes that the fibrillae seen in histological preparations are the results of condensation occurring in the oriented fibrillar substance during fixation. Thus, the fibrils may be considered as being potentially prepared but not bodily pre-established in the living fiber.

In contrast to these negative results we have succeeded in demonstrating the existence of typical and unmistakable fibrillae both in the cell body and in the axis cylinder in living neurons of a warm-blooded vertebrate, the chick, reared in tissue culture.

The fragments used for cultivation consisted of the spinal ganglia of the trunk region of chick embryos incubated for from 8 to 11 days. Younger or older ganglia are not suitable for the purpose. Each ganglion was excised with its connective tissue capsule and explanted into a standard depression slide culture, using as medium a mixture of blood plasma and embryonic extract diluted with tyrode solution. It is essential to spread out the clot as widely as possible, in order to cause the culture to flatten out and thus to improve visibility. As a provision for the later microscopical examination under oil immersion, the cultures were placed close to the cover slip. The cultures were kept for about 2 weeks without transfer.

The behavior of the cultures during the first days is familiar from previous work; nerve fibers, sheath cells and connective tissue cells migrate into the medium. Around the third day the culture is found to be composed of two distinct zones: A central portion, containing the explanted spinal ganglion cells and masses of sheath cells and mesenchyme cells; and a peripheral portion containing the cells and fibers that have migrated into the medium (fig. 1). The circular boundary

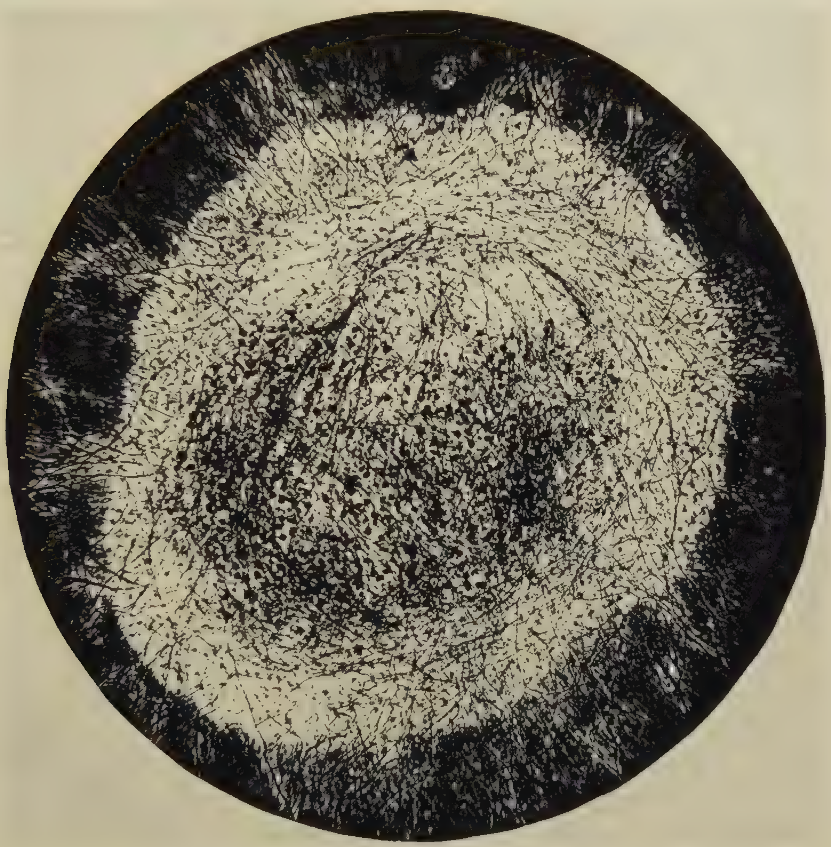


Fig. 1 Fixed preparation of spinal ganglion of 11-day chick embryo, cultivated in vitro for 8 days. Whole mount, Bodian's silver impregnation. The lightly stained inner zone of more dilute plasma contains the ganglion cells (seen as black dots) and the circular plexus of outgrown fibers; the nerve fibers continue radially into the peripheral zone of denser plasma. × 40.

between the two portions is marked by a dense ring of cells and nerve fibers. This concentric wall of fibers surrounding the explant owes its structure to the same factors that were found to be responsible for the formation of a nerve plexus (P. Weiss, '34 b), namely, differences in the mechanical consistency of the medium at the transition from the central to the peripheral area. In the central area, the liquid content of the plasma medium seems to be considerably higher than in the peripheral zone; the difference is illustrated by the markedly lighter stain of the central as compared with the peripheral zone in the silver-impregnated preparations (fig. 1). Although the accumulation of liquid in the area of the original explant was very marked, there was never real fibrinolytic liquefaction of the sort exhibited by brain fragments containing ependymal cells (P. Weiss, '34 a). As the denser peripheral plasma shrinks somewhat and recedes with increasing age of the culture, the central circle is gradually enlarged, making more space available for the individual ganglion cells. Accordingly, beginning with the third day the ganglion cells appear no longer in the original crowded condition but are rather sparsely spaced throughout the central area. In this arrangement they lend themselves readily to microscopic observations with high power objectives.

Most of the cells are bipolar with two axons leaving from exactly opposite poles, but some multipolar cells with one heavy axon and several smaller processes can also be seen. The ganglion cells, varying somewhat in size, are invariably of considerably greater volume than the surrounding sheath cells and mesenchyme cells. During the first 3 days of cultivation the cytoplasm of the ganglion cells appears rather opaque due to the presence of numerous small granules of slightly yellowish tint. Around the third day of cultivation these granules disappear and the protoplasm becomes optically more homogeneous. By this time the nucleus stands out sharply as a clear sphere, structureless in its interior except for a highly refringent, spherical nucleolus.

Four to five days after the explantation the first unmistakable signs of the neuro-fibrillar development appear—as a rule in the part of the cytoplasm surrounding the nucleus. This part appears as a stratum of denser protoplasm with a distinct concentric striation and is set off rather sharply against the more hyaline cortex of the cell. Individual, discrete fibrillae cannot yet be made out in it. The content of the axons is still clear, highly refringent, and homogeneous in ordinary light. Within 1 or 2 days after its first appearance the striation in the perinuclear cytoplasm differentiates into what, henceforth, can be identified easily as a typical neurofibrillar framework in which the fibrillae can be recognized as distinct threads, traceable over considerable distances within the cell. At this stage, the texture of the fibrillar apparatus can be best compared to that of a bird's nest or a reed basket. Its density is greatest near the surface of the nucleus, decreasing gradually toward the periphery of the cell. Generally, the fibrillar basket occupies only part of the cell, and in healthy cells is found in a more or less central position embedded in and surrounded by hyaline protoplasm. About 7 days after explantation the axons are also found to contain distinct fibrils; these appear as continuous filaments with smooth outlines and show no varicosities or other indications of granular structure. In this condition the cells and fibers may remain for several days without marked change.

Only a comparatively small number of fibrillae leave the central fiber basket in a centrifugal direction converging as a bundle upon the mouth of the axon. After entering the axon they continue in their course as independent threads without ramifications or anastomoses. While an exact count of their number has not been feasible, an estimate would place it somewhere around twenty for a larger axon. In studying the behavior of the fibrillar bundles at bends of the axon and at points where obstacles press against its wall, one obtains the impression that the fibrillae have a rather firm and rigid consistency. Satellite cells adhering to the surface of the ganglion cells are a common occurrence.

While it is easy to trace the course of individual fibrils from the axon to the central fibrillar basket, it is plainly impossible to resolve optically the intricate pattern of the fibrillae in the interior of the cell. Observations on the living cell cannot, therefore, decide whether the fibrillae retain their individuality or branch and anastomose among each other in the pericaryon. The latter view is suggested by the study of fixed and silver-impregnated cells; but, as always, in the interpretation of fixed preparations, utmost reserve should be exercised in accepting this evidence. A brilliant stain of the fixed preparations was often obtained with Bodian's ('36) method of silver impregnation, although sometimes local failure of the stain caused interruptions in the course of fibrils. In successful preparations unmistakable branching and anastomosing of the fibrillae of the central network was displayed—similar to the pictures obtained in silver preparations of normal ganglion cells.

In many respects, however, the appearance of the fixed and stained cells is quite different from that of the cells observed in the living condition, and its fibrillar structure is considerably cruder. The discrepancy is particularly striking in earlier stages; for instance, ganglion cells explanted from a chick embryo of 8 days and cultivated in vitro for 5 days, fail to show fibrillar differentiation in the living conditions while, after fixation they display much the same fibrillar structure as would be seen in preparations of older cultures.

Photographs were taken at the height of the fibrillar development: The cover slips carrying the cultures were removed from the depression slides, fastened to ordinary slides by drops of tyrode solution and were photographed immediately. The manipulation caused no noticeable modification in the fibrillar structure. Cultures which were kept for several days after they had been photographed, showed even then no evidence of injurious effects. The photographs, therefore, picture the cells truly and accurately as they appeared in undisturbed life. The photographs were taken in ordinary light without filters, with an oil immersion lens; the low penetrating power of the objective chosen permitted to obtain sharp pictures of individual cells standing out clearly against a blurred background. Ordinary Eastman process plates were used for the negatives, and the sharp contrast between the fibrils and their environment is due merely to the differences in refringency. Figure 2 shows a group of seven ganglion cells with the nuclei and nucleoli in focus. Figure 3 is an enlarged view of two bipolar cells one of which shows both nucleus and nucleolus in focus; both axons can be seen in one cell, while in the other cell the second axon is at a deeper level, out of focus with the others. The appearance of the fibrillar apparatus as illustrated by these photographs is typical.

It must be emphasized that throughout the period during which the fibrillae develop the culture is in full vital activity, displaying progressive phases of growth and differentiation. Measurements of cell diameters on a number of cultures revealed an increase in the average cross sectional area of the cells of from 3 to 27% within 3 days. Since the turgor of the cells has not changed noticeably, it is improbable that the increase in size is due merely to imbibition, and an actual increase in the mass of protoplasm seems indicated. Differentiation of the ganglion cells seems also to proceed along typical lines. All stages of transformation from the bipolar form into the final unipolar, T-shaped, cell known to occur during normal embryogenesis were observed in the cultures. Exceptionally, some tripolar cells with three axons of equal size, leaving at three corners of triangular cells, were noticed.

The continued growth and differentiation of the cells proved beyond doubt that they were living under optimum conditions. Several instances suggested even a certain precociousness of some of the differentiations in vitro as compared with the corresponding events in the organism, but this point is in need of further investigation.

The development of fibrillae does not begin at the same time in all the cells of the explanted ganglion. Even in later stages, when every ganglion cell and fiber is found to be provided with fibrils, some cells show them more distinctly than others. In

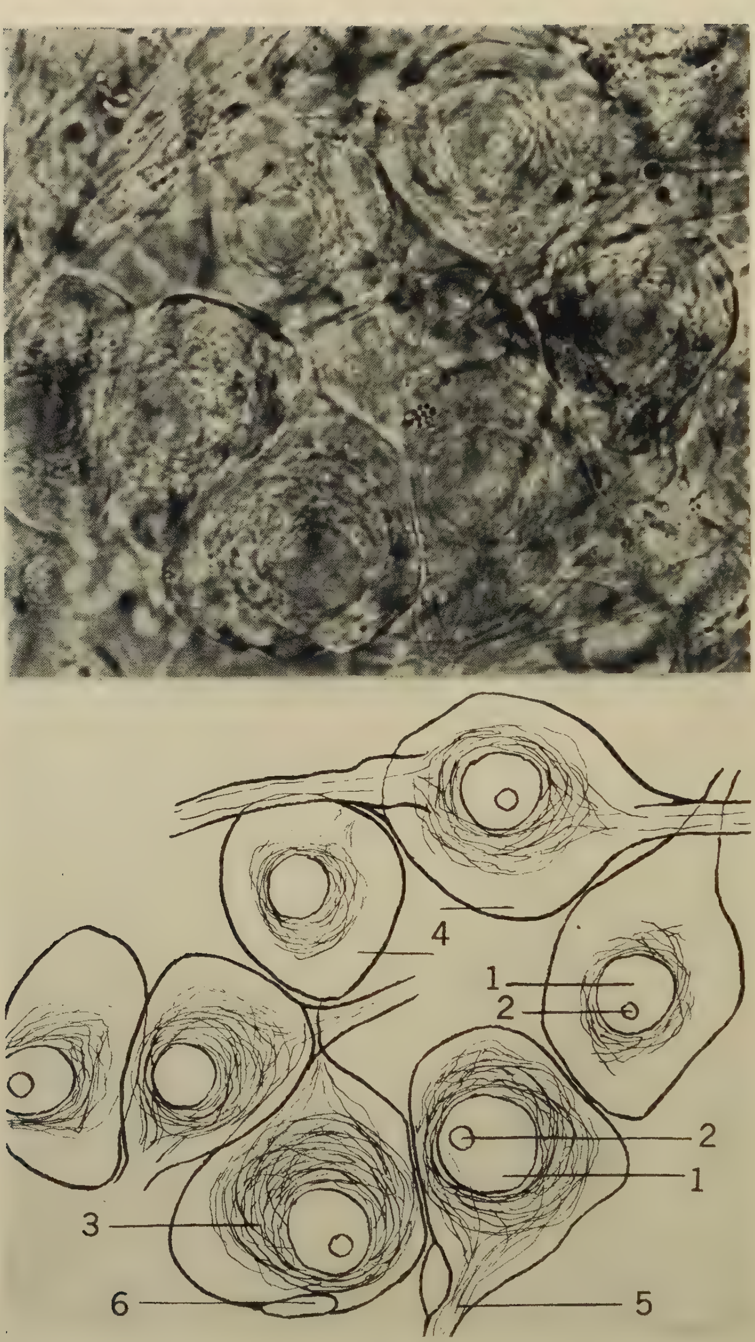


Fig. 2 A group of cells from a spinal ganglion explanted from an 8-day chick embryo and cultivated in vitro for 7 days. a, unretouched photograph of the living culture. × 1300. b, diagram of same. 1, nucleus; 2, nucleolus; 3, fibrillar basket; 4, peripheral hyaloplasm; 5, axon; 6, nucleus of sheath or satellite cell.

general, the fibrillar apparatus is better developed in larger cells than in smaller ones, irrespective of whether the larger size of the cells is due to older age of the embryos at the time of explantation or to prolonged cultivation in vitro. This indicates that the appearance of the fibrillae is correlated with the total age of the cells rather than with the length of their stay in vitro.

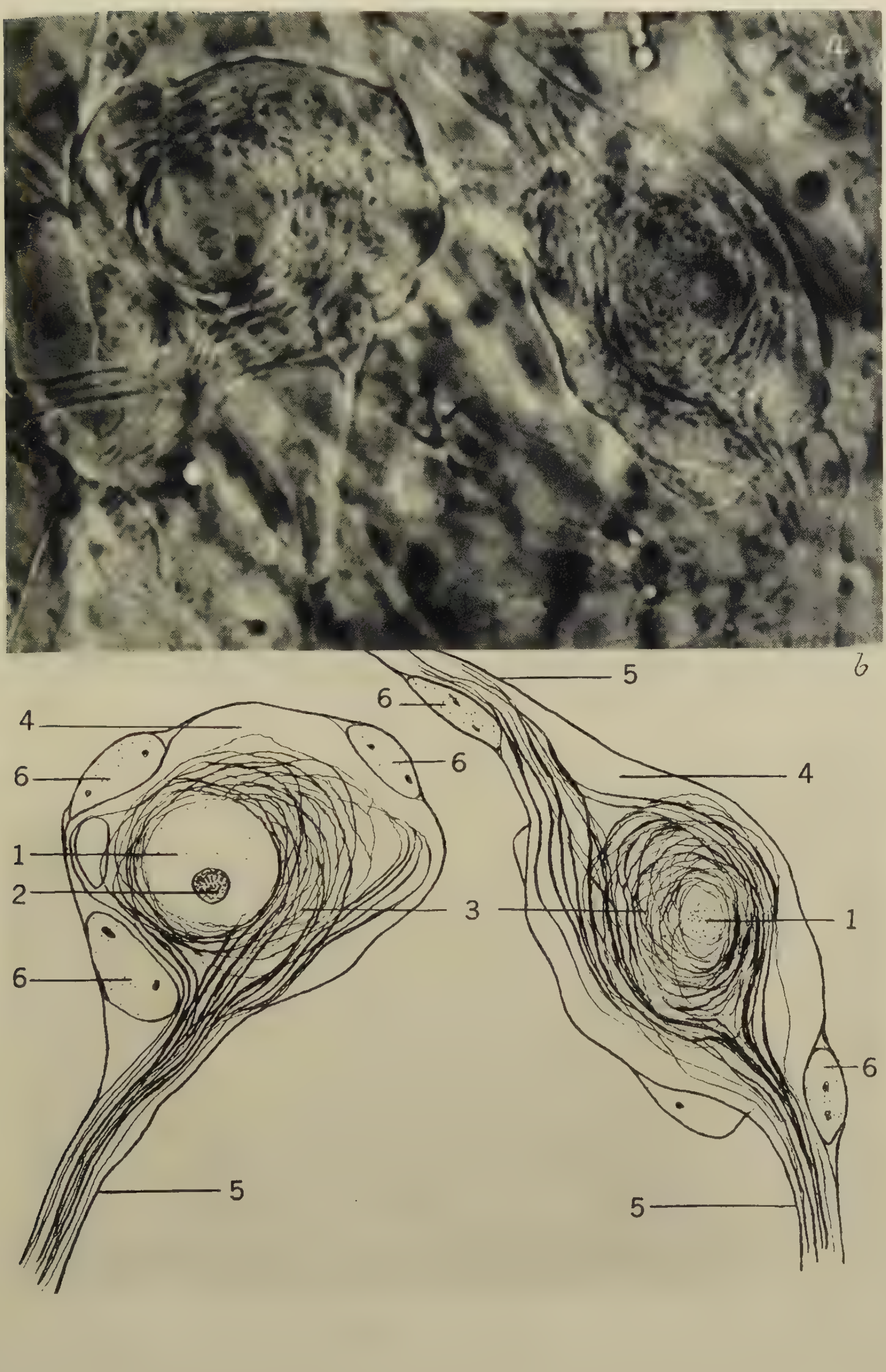
Once the fibrillar apparatus has reached its full development, it retains its character until degeneration of the cell takes place. The time when the first signs of degeneration appear in a culture varies considerably with the conditions of the individual experiment. In most cases the ninth day marked the end of the healthy and normal appearance of the ganglion cells. There are indications, furthermore, that some of the ganglion cells have perished in earlier days of cultivation. Degeneration begins in the cell body with the appearance of clear vacuoles. Many of the small vacuoles become confluent and produce blisters that cause parts of the cell wall to protrude, the protoplasm assumes a granular appearance, and eventually the cell walls collapse. Fixed preparations of degenerating ganglion cells invariably reveal the nucleus lying on the surface of the cell completely outside the fibrillar network which, clumped together, occupies the opposite end of the cell. The fibrils, however, remain distinctly visible even to the last stages of degeneration.

Our observations have convinced us that fibrillae in the strict sense of the word, that is, as discrete, continuous filaments, do exist in the normal living, growing and differentiating spinal ganglion cell of the chick. Their development was followed from its beginning through a transitional stage of fine striation to the final stage of coarse fibrillar bundles. There can be no doubt but that these visible changes express a real progressive formation of the fibrillar apparatus rather than merely a gradual improvement in the visibility of preformed structures owing to optical changes.

The studies on living nerve cells, thus, suggest the following composite picture of the origin of neurofibrils: In the young neurone the neurofibrillar substance is present in a state of high dispersion, the elements oriented more or less parallel to the surface of the nucleus within the soma of the cell, and parallel to the axis of the axon within the latter. A decrease in the dispersity of the system leads to aggregations of the elements into patterns corresponding to their previous orientation. These condensations appear first as faint striations and eventually as fibrillae of microscopic visibility. Only the first stages of this process were seen by Levi and Mossa. Our observations prove that the ultramicroscopic condition is not the final stage of the neurofibrillar substance in the living cell but that a coarse filamentous condition is attained in the normal course of events, provided sufficient time is allowed. The uninterrupted continuity of the definitive neurofibrils, as seen in the living condition, indicates that their coagulation starts from certain centers; and since fibrils, as a rule, appear in the cell body before they can be seen in the axon, it seems reasonable to assume that the centers lie in the vicinity of the nucleus. The action of histological fixing agents imitates in a crude and exaggerated fashion the normal process of fibrillar development.

Of course, the question arises as to how closely the phenomena observed in tissue culture correspond to those occurring in the embryo under normal conditions. One must be aware of the possibility that the circumstances under which the cells were living in the cultures, contrasting with the embryonic environment, may perhaps have vitiated the results by enhancing the fibrillar development to a degree far beyond that normally reached within the organism. There are mainly three factors that could be held responsible for such effects: Injury, dehydration and mechanical stresses. The first factor can be ignored because there was every indication that the cells were unharmed and in excellent condition. Dehydration of the cytoplasm seems contradicted by the continued growth of the cell. And the assumption of major

Fig. 3 Two bipolar cells of ganglion explanted from an 8-day chick embryo and cultivated in vitro for 7 days. a, unretouched photograph of the living cells. × 1650. b, diagram of same, to show the main course of the fibrillae.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tensions acting upon the cells is invalidated by a consideration of the actual conditions: The cells have ample space in which to expand and to move, surrounded by a highly diluted, almost liquid, plasma medium. This fact, in addition to the observation that the axes of the cells may include any angle with the surface of the clot, proves satisfactorily that the horizontal tensions which normally develop in a solid clot adhering to the cover slip were absent within the confines of the circular wall surrounding the central area in which the studied cells were situated. In conclusion, there is no reason to believe that the observed fibrillae were products of the peculiar conditions of the atypical environment rather than of the normal differentiating tendencies of the neurones.

SUMMARY

This study records the development and structure of the neurofibrillar apparatus of spinal ganglion cells explanted from chick embryos incubated 8 to 11 days and grown in tissue culture. Typical neurofibrils, i.e., discrete and continuous microscopic threads, were observed in the living cell. They are arranged in a concentric pattern around the nucleus and radiate from there into the axons. Photographs are presented which display the fibrillar apparatus in the living undisturbed neurone.

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THE SEXUAL CYCLE OF THE CHIMPANZEE[1]

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SIX FIGURES

The use of chimpanzees as biological material in physiological and behavioral studies of sex is a comparatively recent advance. The initiation of systematic studies of sex, like that of many other problems, depended upon the maintenance of anthropoid colonies specifically established for research purposes. Within the past few years there has been a notable increase in the use of monkeys and apes for purposes of sex research, a development which appears to be due in part to the lively interest in endocrine function and the realization that in the elucidation of important problems of human sex physiology these animals present unique advantages over all other forms.

Our present knowledge of the sex cycle in the primates reveals so much similiarity among different genera and species that there is excellent reason for belief that new discoveries regarding one particular species will be of considerable value in supplementing the data obtained from the study of others. The most extensive experimental work on the sexual cycle in infra-human primates has been with the rhesus monkey (Macaca mulatta), the baboon (Papio hamadryas and P. porcarius), and the chimpanzee. A very great advantage over man in the use of these primates is the possibility of satisfactory control of conditions and observations. Rarely with human material is there opportunity for application of objective methods like those of Corner ('23), Allen ('27), Hartman ('32) and others. As a result of these investigations our knowledge of the sexual cycle in the monkey is more complete, precise and reliable than it is for man.

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[1] The writers gratefully acknowledge the assistance of the Committee for Research in Problems of Sex, National Research Council. We desire also to express our thanks to O. L. Tinkelpaugh, M. I. Tomlin, S. Zuckerman and others who are responsible for some of the observations included in this report.

Several reports, descriptive of the chimpanzee cycle or some of its phases in individual subjects, have appeared,[2] but the opportunity is ours to offer for the first time results which have quantitative significance. The present report is a presentation of essential characteristics of the sexual cycle as they have been observed in a colony of mature, captive specimens maintained by the Yale Laboratories of Primate Biology, Inc. at Orange Park, Florida. By February 15, 1936 more than 200 cycles had been observed in thirteen females. This colony has provided exceptional material and opportunity for research on problems of sex. As it is used primarily for breeding purposes it is possible to observe and record the process of sexual maturation from early puberty to completion of reproductive function in the individual.

Subjects and conditions of observation. Table 1, a list of the eighteen subjects used in this study, shows sex, birth dates (hypothetical for all except Cuba), brief description of sexual status, reproductive history of the females and the number of cycles observed for each. By the criterion of motile sperm, all males were sexually mature throughout the period of observations; while the impregnation criterion had been satisfied even by the youngest male (Al) by October 1935. The reproductive history of the females shows that four of them have not shown evidence of fertility, although they have participated in numerous matings. One of these (Bentia) is still very young and her cycles irregular. Mimi also has shown considerable irregularity although she has been menstruating since July 1932. Records of these two animals were not used in determining cycle length for the group. May has recently experienced normal pregnancy and parturition. Nira's cycles are quite regular although several days above the average in length. Her behavior in the mating situation must be classified as abnormal, inasmuch as she will not ordinarily accept a male under conditions which we have found to be satisfactory for other subjects.

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[2] We omit discussion of these observations—except as they may be referred to in connection with specific topics which follow—since they have been reviewed by several authors during recent years, Bingham ('28), Yerkes and Yerkes ('29), Zuckerman ('32), Tinklepaugh ('33), Schultz and Snyder ('35).

TABLE 1

List of chimpanzee subjects

NAME AND NUMBER	SEX	BIRTH-DATE	SEXUAL STATUS	REPRODUCTIVE HISTORY [1]		NUMBER OF CYCLES OBSERVED
				Births	Miscarriages	
Bentia (16)	F	1926	Recently mature	0	0	7
Cuba (46)	F	Mar. 24, 1926[2]	Recently mature	1	0	16
Dita (40)	F	1920	Long mature	2	1	18
Fifi (38)	F	1918	Long mature	3	0	4
Josie (30)	F	1922	Mature	1	1	8
Lia (56)	F	1924	Mature	0	1	20
May (32)	F	1925	Recently mature	0	0	16
Mimi (44)	F	1923	Mature	0	0	25
Mona (36)	F	1913	Long mature	5	1	16
Nana (6)	F	1921	Mature	2	0	15
Nira (64)	F	1926	Mature	0	0	17
Pati (42)	F	1920	Long mature	2	1	23
Wendy (4)	F	1923	Mature	1	0	22
Al (9)	M	1927[3]	Juvenile			
Bill (1)	M	1922	Young			
Bokar (5)	M	1925	Young			
Jack (17)	M	1920	Mature			
Pan (3)	M	1922	Mature			

[1] As of July 1, 1935.

[2] Actual birth date. All other dates are hypothetical.

[3] Our original estimate was 1928. In 1935, this was revised to 1927.

Objective methods of recording the sexual status of our subjects have been recognized as a primary requisite for all studies of sexual and reproductive behavior. Our present system of observation provides an accurate record of the onset and duration of menstrual bleeding, appearance of genital swelling and subsequent variations, size and appearance (color, vaginal secretion, epithelial sloughing) of the maximal swelling, beginning of detumescence, quiescent phases, etc. Beginning with the device developed and described by Tinkelpaugh ('30) for the measurement of irregular contours, attempts have been made to find a means of measuring the volume of the genital area. The problem has not yet been solved satisfactorily, but we have minimized the deficiency through regularity of examination, by experienced observers intimately acquainted with each individual.

The sexual cycle may be roughly divided into phases of genital swelling and absence of swelling. Complete detumescence or absence of swelling we have designated as the 'permanent residual' condition. (Symbolized in daily records by 'PR.') Size of the external genitalia and surrounding areas varies from this residual, detumescent condition to a maximum which is relatively constant for a given female from one cycle to another. The range then from no swelling to maximum may be represented by a fractional scale from 0 to 1, increases being indicated by a positive sign; decreases by a negative sign. Ordinarily the divisions are by quarters, although occasionally very slight but definite changes from the PR condition are recorded as $+\frac{1}{8}$ and likewise a slight decrease from the maximum as $-\frac{7}{8}$. The observer, at the time of daily inspection, notes the condition of a given female and enters the record in his daybook. The records shown in table 2 illustrate the use of the fractional scale. In the first cycle the animal was menstruating on the first 4 days. On the fifth to the thirteenth days a quiescent, detumescent condition prevailed. Slight swelling appeared on the following day, increased to $+\frac{1}{4}$ and $+\frac{1}{2}$ and reached maximum on the seventeenth day. As may be seen in some of the other cycles the increase and decrease in swelling is not always as rapid as in this one. The more gradual the change, of course, the more fractional symbols are required to represent it.

Variations in the volume of the sexual skin are of two kinds: a) Those between the detumescent and maximal conditions of a given cycle, and b) differences in the maximum size displayed by various individuals, and, to some extent, by the same individual at different times. In order to indicate the relative size of the maximum another scale from 1 to 4 is used. Thus a given animal may exhibit a small maximum of 1 while another individual shows a large maximum of 4. Each rating is assigned with regard to differences in size and body weight, so that a maximum of 2 for a large animal may correspond to 2½ or 3 for a smaller one. All subjects are inspected daily at approximately the same hour of the morning, but if an animal is approaching a critical phase of the cycle (end of maximal swelling or onset of bleeding) she may be observed more frequently. In some individuals with a short or scant menstrual flow the more frequent observations are important. These daily inspections are facilitated by having all subjects trained for posterior presentation to the observer at his command. This cooperation is essential for satisfactory inspection and reliable observations. The habit is easily learned and is reinforced by food reward.

The daily observations are used in making the permanent tabular and graphic records of sexual status. Charts for each animal show the onset and duration of menstruation, the time and extent of genital swelling, dates of matings, social relations, weight, illness, participation in experiments and other facts which may be related to the sexual status. Figure 1 is a reproduction of Wendy's record for the year 1932. The numbers just below the sexual cycle curve indicate the length of each cycle and (in parentheses) the number of days before and after the beginning of detumescence. The tabulated data are arranged chronologically and according to days of the cycle. Thus the sexual status of an animal on any given date or any day of the cycle may be ascertained readily. Table 2 is a sexual status record for Wendy arranged according to days of the cycle. In this form it is possible at a glance to compare the cycles in respect to total length and component parts, or to compare the status on any given day throughout several cycles. Dates to the left refer to the first day of each cycle.

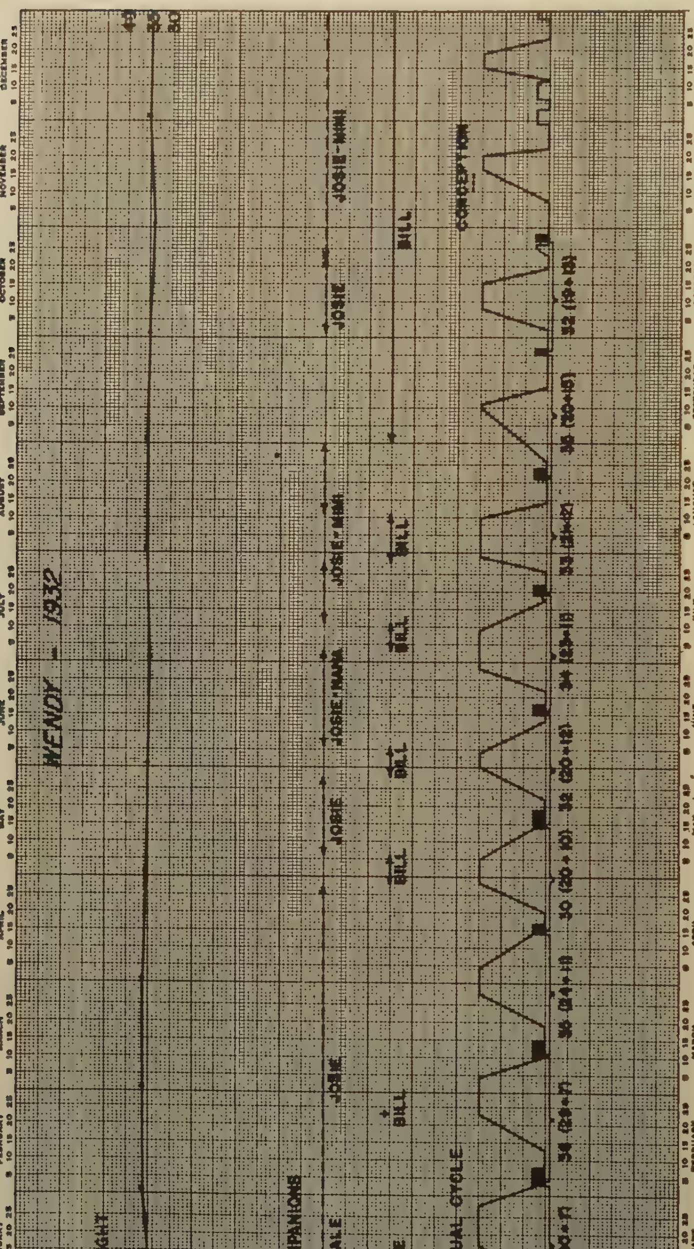

Fig. 1 Graphic sexual status record of an individual for a period of 1 year, showing monthly weights, cage mates, curve of genital swelling, and menstruation. The numbers below the sexual cycle curve give the total cycle length, and the number of days before and after the first day of detumescence.

TABLE 2

Nine consecutive cycles for Wendy tabulated according to days of the cycle

DAYS OF CYCLE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1934 SEPT. 17	M	M	M	M	•	•	•	•	•	•	•	•	•	+1/8	+1/4	+1/2	m	m	m	m	m	m	m	m	m	m	m	m	-1/2	-1/4	•	•	•	•	•	•	•	•	•	
OCT. 26	M	M	M	•	•	•	•	•	•	•	+1/4	+1/4	+1/2	+3/4	+3/4	m	m	m	m	m	m	m	m	m	m	-3/4	-1/4	•	•	•	•	•	•	•	•					
NOV. 30	M	M	M	•	•	•	•	•	•	•	•	•	+1/8	+1/4	+1/4	+1/2	+3/4	+3/4	m	m	m	m	m	m	m	-3/4	-1/8	•	•	•	•	•	•	•	•	•	•			
1935 JAN. 6	M	M	•	•	•	•	•	+1/8	+3/4	m	-3/4	m	m	m	m	m	m	m	m	m	m	-3/4	-1/2	♂ •	•	•	•	•	•	•										
FEB. 5	M	M	M	•	•	•	•	•	•	+1/8	+1/4	+1/4	+1/2	+1/2	+1/2	+3/4	m	m	m	●♂ m	m	m	m	-3/4	-1/2	-1/8	•	•	•	•	+1/4	+3/4	+3/4	-1/2	+3/4					
MAR. 12	$M^{-1/2}$	$M^{-1/8}$	•	•	+3/4	+3/4	m	m	m	m	m	m	m	m	m	m	m	m	-3/4	-1/4	•	•	•	•	•	•	•	•	•	•	•									
APR. 12	M	M	M	•	•	+1/4	●♂ +1/2	+3/4	m	m	m	m	m	m	m	m	m	m	♂ m	m	-1/2	-1/4	•	•	•	•	•	•	•	•	•									
MAY 13	M	M	+1/4	●♂ m	m	m	m	●♂ m	m	m	m	●♂ m	m	m	m	m	●♂ m	-3/4	♂ •	•	♂ •	♂ •	•	•	•	•	•	•	•											
JUNE 11	M	M	$M^{+1/8}$	$M^{+1/4}$	+1/8	●♂ +3/4	m	●♂ m	m	m	m	m	m	m	m	m	m	●♂ m	-1/2	●♂ -1/4	♂ •	♂ •	♂ •	•	•	•	•	•	•	•	•	+1/8	+1/8	+1/8	•	TO PARTURITION ⟶ •	+1/8	+1/4	+3/4	+3/4

FRACTIONS WITH + SIGN INDICATE RELATIVE SIZE OF INCREASING SWELLING
FRACTIONS WITH – SIGN INDICATE RELATIVE SIZE OF DECREASING SWELLING
M = MENSTRUATION
m = MAXIMUM SWELLING
• = NO SWELLING, COMPLETE DETUMESCENCE
♂ = MATING WITHOUT COPULATION OR INSEMINATION
●♂ = MATING WITH INSEMINATION

Characteristics of the sexual cycle. In the following pages we shall describe in some detail the constituent phases of the sexual cycle in the chimpanzee. The term 'sexual cycle' will be used frequently as denoting the series of external, macroscopic processes and conditions, beginning with the endometrial or 'menstrual' bleeding. With exception of this period of bleeding the principal phases of the cycle are represented by the presence and absence of genital swelling and intermediate conditions. The relation of the sexual cycle, as we define it, to uterine and ovarian cycles is inferential. Ovulation, for example, may or may not occur.

Discrepancies among the few published accounts of the sexual cycle in the chimpanzee most frequently involve temporal and physical relationships of different phases and events of the cycle. One may find statements to the effect that mestruation and genital swelling are contemporaneous, that the former immediately precedes or follows the latter, that the menstrual cycle is a lunar month in length, and so on. It is hoped that the description which follows may contribute to a clearer understanding of the chimpanzee cycle; but we do not insist that all observations must agree with ours or be in error, for we are well aware that differences in care, handling and use of animals may produce variations from our norms.

The sexual cycle of the chimpanzee averages approximately 36 days, a week longer than the cycle of man and monkey. Figure 2 shows a frequency distribution of the length of 164 cycles in eleven presumably normal and mature animals.[3] In computing the group average the records of Bentia (seven cycles) and of Mimi (twenty-five cycles) were not included for reasons given in discussion of table 1. We also omitted from the original total of 207 cycles: Three of 46, 54 and 56 days because of illness during the cycle; six cycles (46, 49, 65, 69, 73 and 161 days) in which the cause of irregularity was unknown; and two cycles during the lactation interval. Of those included, a few of the longest ones may also have been affected by abnormal conditions unknown to us. In answer to the question which may arise concerning rejection of certain cycles we should like to say that the ones omitted are useful for the purpose of studying gross variability under all conditions; but they should not be used in determining an average since their effect would be to produce a measure not typical of any individual. It should be noted that all cycles omitted are considerably above any of the individual averages.

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[3] These are identical with the 158 cycles used in a previous summary report (Yerkes and Elder, '36) except for the inclusion of two additional cycles for Dita and four for Fifi.

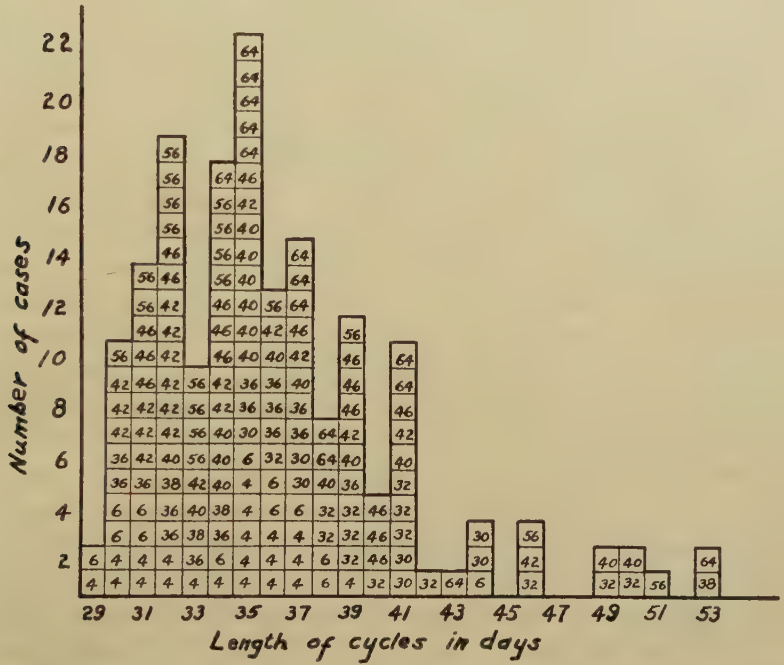

Fig. 2 Frequency distribution of the lengths of 164 sexual cycles. Numbers within each square refer to individuals as listed in table 1.

By referring to the small figures in each square, which indicate the numbers of the animals showing cycles of the lengths represented, one may obtain an idea of individual variability. The number of cases observed hardly seems to justify a more complete description of individuals in terms of measures of dispersion. Figure 2 indicates that variability of the chimpanzee cycle is comparable with that of women (King, '33; Fluhmann, '34; Latz and Reiner, '35).

The standard deviation of the group average for chimpanzees is 4.82, but it should be noted that included in the group are three females with long cycles ranging upward from 34 days and averaging about 40 days. As we accumulate more observations we may find reason for a more precise classification into long- and short-cycle types. Other observers have reported to us the occurrence of regular cycles of more than 40 days. The skewness of the distribution of figure 2 is due in part to the long-cycle individuals, but also to the skewness of individual distributions. There are no extremely short cycles to compensate for the occasional long ones.

Variability of the cycle length in the individual is determined by many conditions. We list without discussion such influences as illness, abortion, endocrine disturbances, nutrition, climate, social relations, frequency of matings, etc. Species, racial, or individual characteristics probably account for the consistently short- and long-cycle types.

Periodic or menstrual bleeding in chimpanzees occurs typically at about 35-day intervals and continues for 1 to 7 days varying according to the individual. Averages for eleven animals range from 2.1 to 4.5 days (compare Schultz and Snyder, '35). Intermittent bleeding is not uncommon but seems to be an individual and not a group characteristic. Of twenty-four observed instances in thirteen subjects, fifteen were in two individuals (Cuba, Mimi). The catamenial flow typically appears in the absence of marked genital swelling. Statements that it occurs during the period of maximal swelling are certainly erroneous, either indicating some functional abnormality or hemorrhage of entirely different origin. It is especially important to point out, however, that a moderate degree of enlargement is sometimes present for 2 or 3 days preceding, but rarely continuing through, the period of bleeding. We consider this a significant phenomenon especially in connection with the endocrinological antecedents of menstruation. Since it has been shown that the follicular hormone oestrin is produced by the corpus luteum this slight swelling may represent some acceleration of function in this body. Or, again, the premenstrual swelling may indicate the beginning of another follicular cycle which is promptly repressed by those elements which induce bleeding. Siebke ('30) and Zondek ('31) found oestrin in the urine of women throughout the menstrual cycle with highest concentration during the premenstrual period. Allen, Diddle and Elder ('35) found comparable quantities in urine and placentae of chimpanzees. A further quantitative study by Allen, Burford, Diddle and Elder ('36) indicates that greatest amounts are excreted during the middle of the cycle and just before or during the menstrual period. (See also Engle, Smith and Shelesnyak, '35, for discussion of relation of oestrin and progestin to menstruation.)

It is possible that the false identification of the premenstrual swelling as mid-cycle swelling is responsible for the impression that bleeding accompanies the latter. Although premenstrual swelling may extend into the period of bleeding, it ordinarily does not, and because of its transient character it should not be confused with the much more conspicuous mid-cycle swelling. Moreover, we have observed only about twenty instances of the phenomenon and, therefore, may not regard it as a typical feature of the cycle even in individual subjects. One of these instances is represented at the end of the fifth cycle shown in table 2.

Following the period of bleeding the sexual skin usually remains quiescent for several days. The length of this interval of inactivity depends on the total cycle length, individual characteristics and various social and environmental influences. Averages for eleven subjects range from 5.1 to 13.7 days, including the menstrual period. In a few cases, however, swelling has followed bleeding immediately.

Involving at first the labial and circum-anal areas, the swelling gradually extends anteriorly through the pre-pudendal region and laterally to the callosities, attaining its maximum size ordinarily not later than the fifteenth day of the cycle. There is much variation in the gross appearance of the maximal swelling. In some chimpanzees, especially in recently matured specimens, the enlarged perineum has well-defined boundaries and stands out prominently, while in others the hypertrophy may involve surrounding areas and be less distinct in outline. Scaling and sloughing of the surface epithelium, a feature of the baboon cycle which Zuckerman ('30, p. 714) regards as heralding retrogression of swelling, frequently is seen during the maximal period in chimpanzees, but thus far it has not proved to be a particularly reliable sign of detumescence. Its appearance depends greatly upon the extent and duration of swelling. For example, small fluctuations will cause the surface to crack and peel. If a constant degree of tension were maintained until detumescence began, then the scaling might possibly be seen only at this time.

Accurate measurements of the genital swelling are not available. Approximately determined dimensions on one animal (Nira) indicate a volume, at maximum, of about 1400 cc. The maximum in this individual nearly, if not actually, represents the maximum for the colony. Color changes, which occur throughout the cycle, seem to be much less significant in chimpanzees than in monkeys. Color may appear in various shades of pink, from pale to deep rose, and may be seen throughout the cycle, with or without apparent change. It may be most prominent during maximal swelling or after detumescence begins.

A notable feature of the genital swelling is the rapidity of transfer and absorption of this relatively enormous mass. While the rate of increase from no swelling to maximum may be quite rapid, especially under the influence of continuous sexual excitement, the detumescent phase may be as short as 48 hours in some animals, with a large percentage of the decrease occuring in the first 24 hours. Histological studies of Zuckerman and Parkes ('32) show that the swelling in the baboon is a result of an accumulation of intercellular fluid rather than cellular enlargement. Presumably the tissue changes in chimpanzees are of a similar nature.

It has been demonstrated by several investigators that experimental injection of oestrin into ovariectomized monkeys will produce enlargement and coloration of the sexual skin. Histologically such swelling is the same as that found in a normal animal. There is little doubt that this hormone is also responsible for the sexual skin swelling of chimpanzees although as confirmation we have only the evidence of Zuckerman and Fulton ('34) on the effect of oestrin injection in an adolescent, ovariectomized animal. Even though the subject was in extremely poor physical condition during the experimental period, some indications of a positive reaction were obtained. Ovariectomy performed on monkeys during the phase of maximal swelling results in prompt subsidence of the sexual skin, an effect which likewise follows withdrawal of experimental injection of oestrin. In the normal animal the progressive enlargement presumably follows the process of follicular maturation which eventually culminates in ovulation and abrupt change in endocrine relationships. On the basis of such considerations as the foregoing, Zuckerman concluded that ovulation is reliably indicated by detumescence. The relation between external and ovarian events in the chimpanzee will be discussed later. Here it is necessary for the completion of our description of the follicular phase and the period of swelling to state that detumescence begins between the twentieth and thirty-first days in 75% of our cases with an average of approximately 26 days.

The relationship between the sexual cycle and oestrus constitutes a topic which does not fall in the compass of the present report. This and other aspects of sexual behavior are being presented elsewhere (Yerkes and Elder, '36 b).

Menstruation and genital swelling during pregnancy and lactation. During a 6-year period these laboratories have accumulated records of twenty-five pregnancies, definitely established by normal gestation and parturition or abortion. Since the publication of a recent statement by us (Elder and

Yerkes, '36) that menstruation is absent throughout the entire period of gestation we have observed one instance of bleeding after a verified fertile mating. Although we regarded this observation as one of implantation bleeding, it occurred 18 days after the last mating at exactly the time when menstrual bleeding was anticipated. The discharge was abundant and could not be distinguished in appearance from menstruation. This one case, however, does not alter the correctness of our original statement in its general application. Cycles of swelling, usually irregular, do occur during the early months of pregnancy, but the swelling does not reach the dimensions characteristic of the non-pregnant cycle.

Quiescence of the sexual skin and amenorrhea are characteristic also of the lactation period, although swelling will appear occasionally in some females especially after prolonged nursing. Twice we have observed bleeding of the menstrual type.

It is important to note the contrast between the detumescent condition of the non-pregnant, mature female and that of the lactating animal. In the latter the perineum is quite atrophic and inactive, smaller than the detumescent condition of the non-pregnant female, and scarcely more conspicuous than in the immature female. This condition persists without change until a few days after the infant is weaned when a sudden transformation occurs. The pinched folds of the vulva become less well defined and the entire area acquires a freshened, vitalized appearance. This change, however, even though it involves some increase in size, may not properly be termed genital swelling of the type we have described, but instead represents the permanent residual condition referred to on page 122. It may reflect an internal adjustment preparatory to return of ovarian cycles, which have been inhibited for several months by the presence of lactogenic hormones.

Time of ovulation in chimpanzees. Much of the recent sex research with mammals is directly or indirectly pointed toward the determination of ovulation time. This problem is a part of the sex research program at the Yale Laboratories, and has already yielded results which appear to justify the presentation of a few preliminary statements. Although we anticipate early publication of a more complete account than we now are prepared to offer, we believe that any reliable information on ovulation time in primates is worthy of prompt announcement. Interest in the question, as it applies to man, is widespread, but facts are few notwithstanding that much effort has been directed toward a solution of the problem.[4]

From the relatively few choices of practical methods for determining ovulation time in chimpanzees the controlled mating procedure was selected. This method consists simply of permitting a single, effective, sexual contact for a limited period of time within the cycle and subsequently determining whether or not fertilization has occurred. Before the procedure begins to yield positive results, however, much preliminary work is necessary. A subject may be allowed to mate only once during a cycle which means that several weeks are required for each trial. As an aid at the outset to the selection of appropriate days for mating were the records of sexual behavior, from which it was possible to make a few general predictions. For example, there were several instances of failure to mate during early and late phases of the cycle and when mating did occur only at these times it was, without exception, infertile. It was with such information as this that the task of excluding the non-fertile days began. The procedure originally adopted began with a series of mating opportunities using a given female at a late, probably infertile period of the cycle, and gradually advancing (usually by 1 day) the date of mating in succeeding cycles. Thus, for example, a female was used on the twenty-fifth day of a given cycle, the twenty-fourth day of the next, and so on until a fertile mating occurred. The chief reason for this particular approach was to avoid the contingency of too many contemporaneous pregnancies. Later it became desirable to conduct the mating observations on the most probably fertile dates of the cycle.

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[4] There are many recent articles which reflect the controversial nature of this subject, of which the excellent review, interpretation, and summary by Hartman ('36) is an authoritative account of the present status of the problem.

Almost nothing was known of ovulation time in chimpanzees when our program of controlled matings was begun in 1934. Results obtained within the first year indicated that ovulation occurred at, or shortly after, the mid-point of the cycle (Elder and Yerkes, '36). Additional data enabled us (Yerkes and Elder, '36 a) to suggest the sixteenth to twenty-fourth days of the cycle as the probable limits of fertility.[5] At that time we presented cycle norms for ten individuals and gave the average time of ovulation as the eighteenth to nineteenth day. We have little to add to this general statement except to emphasize the influence of cycle length upon ovulation. For the average 35- or 36-day cycle the eighteenth day probably is the optimal time for fertile mating, but is much too early for the longer cycles. One of our pregnancies, for example, followed a single isolated mating on the twenty-fourth day of the cycle. Cycles of this individual average over 40 days in length.

In order to eliminate the differences which arise as the result of various cycle lengths it appears simpler to date matings from the beginning of the following period of bleeding or, in case pregnancy intervenes, from the next 'missed' period. This date is predicted from the subject's previous record. Located in this way, all of our most probable dates of fertile mating fall within 15 to 19 days prior to the next anticipated menstruation.

Only those mating opportunities in which copulation occurs and insemination is verified by obvious external sign or microscopic examination of vaginal content furnish direct evidence on the time of ovulation. However, from the biological point of view, all other cases have some relevance. There is ample evidence for mammals in general that the absence of copulation during mating opportunities is highly correlated with non-fertile periods. Conversely, positive sexual responses occur near the time of ovulation—in many species, at this time only. The copulative period in chimpanzees is not restricted to a single day, but the distribution of positive responses reveals a definite periodic increase in activity.

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[5] In a recent unauthorized publication of data on the gestation period and the menstrual cycle taken from the Yale Laboratories, M. I. Tomilin ('36) places the time of ovulation at the middle, or slightly prior to the middle, of the cycle. This statement is admittedly based upon analogy and the assumption that the chimpanzee occupies an intermediate place between man and monkey. Unfortunately, Mr. Tomilin's calculations neglect differences in cycle lengths and therefore his statement that "it seems justifiable to place ovulation at the 16th, or the latest, 17th day" may be in error by as many as 7 or 8 days for long cycle individuals.

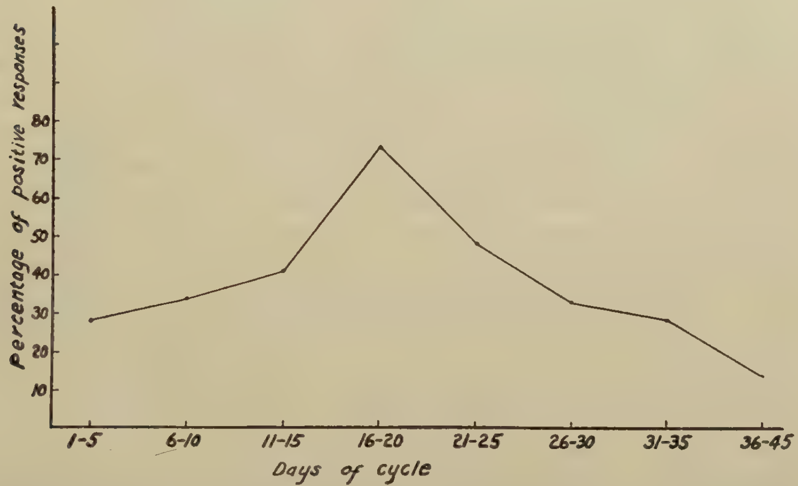

Fig. 3 Percentages of positive responses (copulation) during various 5-day periods in the cycle, showing a maximum of 73% within the 16- to 20-day period. A total of 289 mating opportunities are represented, of which 175 were positive with distribution of percentages as shown.

Figure 3 shows the percentage of such responses occurring in 5-day intervals. The figure comprises the records of 396 mating opportunities involving twelve females and three males. Only the data for one female (Nira) have been omitted because of persistently atypical behavior in the mating situation, and the fact that practically all observations were obtained with a young and inexperienced male. The figure shows a maximum percentage of positive responses between the sixteenth and twentieth days following menstruation.

Actually the highest percentage occurs at the mid-point of this interval or on the eighteenth day. Days 19, 20, 17 and 24 are next in order of positive response percentages.

If relative frequency of copulation is a criterion of the ovarian cycle then these observations strongly indicate that ovulation occurs within the sixteenth to twentieth day period. It should be remembered that the records for twelve individuals, irrespective of cycle length, are represented in this figure. This fact prejudices the results to some extent, because of the high probability that ovulation time is not independent of total cycle length, and suggests the alternative method of reckoning from the beginning of the following cycle. Another essential consideration is the individuality of the male responses. All males used by us showed a general tendency toward greater frequency of copulation with females who were near the middle of their cycles, but two of the males (Bokar and Jack) frequently mated in early and late phases, even during the detumescent periods. Results of matings involving these males are entirely responsible for the positive responses between days 1 to 10 and 25 to 45. That is to say, the third male, Pan, in seventy mating opportunities, has never copulated with any female on these days of the cycle. (For a more complete account of the male response see Yerkes and Elder, '36 b.) His behavioral record strongly supports our opinion that ovulation occurs in chimpanzees within a restricted period near the middle of the cycle. It should be added that this male has thoroughly demonstrated his abilities as a breeder.

As corroborating our interpretation of figure 3, some recent observations of Ball and Hartman ('35) show that increase in copulation and other sexual activity in the monkey immediately precedes ovulation. It would be surprising indeed if the behavior records with their mid-cycle peaks of activity had some different significance for chimpanzees. If one is not to infer that ovulation follows this sharp rise in copulation frequency, as it does in monkeys, then what remains as a more plausible conclusion?

Relation of genital swelling to ovulation and menstruation. We have mentioned Zuckerman's hypothesis that detumescence of the sexual skin is a sign of ovulation in the baboon. Does such a relation exist in chimpanzees? How promptly and in what manner is the rupture of a follicle reflected in the external appearance of the swollen genital region? If we assume that signs of detumescence are as readily detectable in chimpanzees as in baboons and that the viability of ejaculated sperm is comparable, then detumescence in the former does not follow ovulation as promptly as Zuckerman's statements would indicate. As evidence for this statement is the fact that some of our fertile matings occurred as many as 6 days before the first sign of detumescence.

It is not necessary to know the exact time elapsing between ovulation and detumescence before we may speak of their relationship. We may be reasonably sure that the maximum swelling is a counterpart of the follicular phase of the ovarian cycle and begins to wane either immediately or soon after the culmination of this phase. As a point of reference in the cycle the beginning of detumescence ordinarily is as definite as menstruation. It divides the cycle into two parts, the first of which is roughly twice as long as the second. There are certain relationships between these two parts of the cycle which we believe to be of fundamental importance for the adequate comprehension of the sex physiology of the primates. We invite particular attention to the constancy of the second part of the cycle (part B) as compared with the first part (part A). The total length of the cycle is relatively independent of part B; the variability of the whole is a function of the variability in part A.

The results of this analysis of the chimpanzee cycle are in agreement with the opinion of many authorities, namely, that in women only the post-ovulational portion of the cycle is relatively constant for all individuals, and, hence, the contention that ovulation should be reckoned not from the last menstrual period but from the beginning of the next. This apparent conformity between man and the chimpanzee does not extend to the monkey. With the cycle divided accurately into pre- and post-ovulatory portions Hartman ('33) correlated these parts with the total cycle lengths. Using fifty-three cases he found a high positive correlation between the post-ovulation interval and the total cycle length. That is to say, the variability in the total cycle length is attributable to variability of the post-ovulation portion. Our data for chimpanzees are not exactly comparable with those of Hartman, since he uses ovulation time as a dividing point while we use the first day of detumescence. If, however, we use Hartman's method of presentation, constructing scatter diagrams to show the correlations, we obtain results quite different from his. Figure 4 presents scattergrams of part A and part B plotted against corresponding cycle lengths. The product-moment correlation coefficients are as follows:

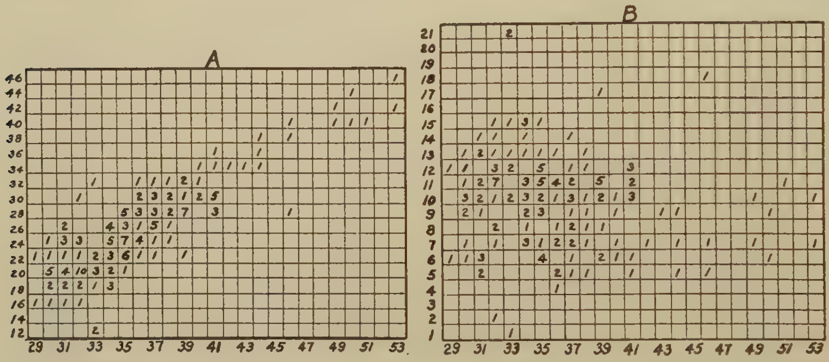

Fig. 4 Scattergrams showing the pre-detumescent period (part A) and the period from beginning of detumescence to the end of the cycle (part B) plotted against the total length of 164 cycles.

Between part A and total cycle, r = 0.863 ± 0.013
Between part B and total cycle, r = —0.142 ± 0.052
Between part A and part B, r = —0.604 ± 0.034

It will occur to the reader that these coefficients are spuriously high because of the fact that they are obtained by correlating a part with its whole, but this does not invalidate the procedure for the purpose for which we have used it, merely to show the presence of different relations.

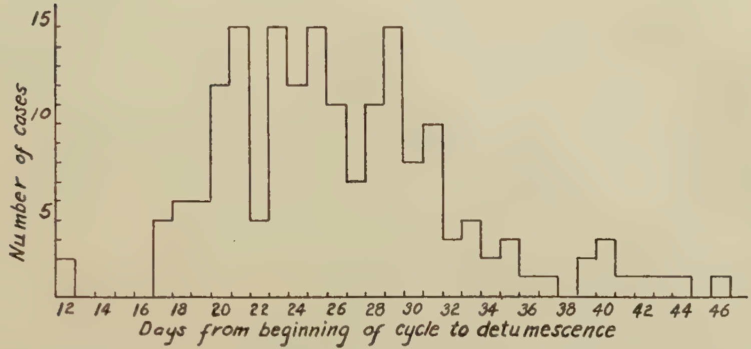

Fig. 5 Distribution of the lengths of pre-detumescent periods in 164 cycles, showing much variability in time of occurrence of the first day of detumescence when located in respect to the beginning of the cycle.

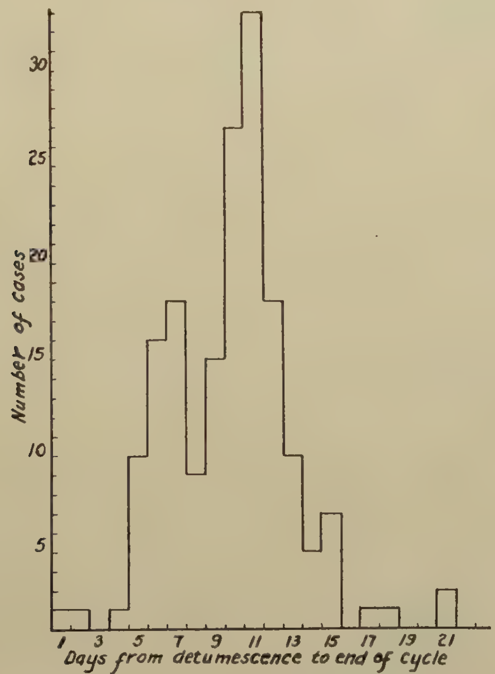

Fig. 6 Distribution of the lengths of the period from first day of detumescence to the end of the cycle, showing relative constancy of this critical day when located in respect to the onset of the following menses.

The extent of variation in the two parts of the cycle is more effectively shown in figures 5 and 6 which present frequency distributions of the length of each part. The facts represented by these two figures again indicate that events occurring during the latter part of the cycle will be more accurately located if calculated from the next anticipated menstrual date instead of the first day of the cycle (Ogino, '30; Knaus, '33; Latz and Reiner, '35).

Some of the observations we have presented are less conclusive than provocative. Much more time can be given profitably to the acquisition of supplementary material of the same kind as well as a better knowledge of the physiology underlying the more readily observable physical and behavioral sequences which we have described. We can see now enough similarity—and dissimiliarity—in monkey, chimpanzee and man to guide our investigations into productive channels and to make the course exceptionally interesting.

SUMMARY

This report is a description of the general features and certain specific characteristics of the sexual cycle as observed in a colony of thirteen mature female chimpanzees. The sexual status of all subjects used in the investigation was determined by means of systematic daily inspections for which the animals were trained to cooperate by posterior presentation to the observer.

The sexual cycle of the chimpanzee is similar to the cycles of other primates although the average cycle is 6 to 7 days longer than in man and the macaque. The average chimpanzee cycle is approximately 36 days with a median and mode at 35 days. Variability of the cycle in man and the monkey approximates the normal curve whereas the distribution of cycle lengths in the chimpanzee is skewed to the right, a characteristic which appears to be due in part to individual as well as group variability. Factors affecting the cycle tend to lengthen rather than shorten it. Cycles which may be considered normal range from 29 to 53 days. None shorter than 29 days has been observed.

The typical cycle begins with a 3-day menstrual period, followed by 5 or 6 days during which the external genitalia is quiescent. Enlargement of the sexual skin appears about 10 days after the onset of bleeding, reaches a maximum within a week, and remains in this condition for 10 or 12 days. Detumescence begins on the twenty-sixth day, as average for a group of eleven females, but this date depends greatly upon the individual and the total cycle length.

From the initial results of a systematic program of controlled matings we adopt the tentative hypothesis that the period of greatest fertility in the chimpanzee lies between the sixteenth and twentieth days in animals with 33- to 37-day cycles, but as late as the twenty-fourth day in the longer, 40-day cycles. This conclusion is based upon the following evidence: a) Records of isolated fertile matings; b) behavioral records which show a maximum percentage of copulative responses, in respect to the total number of mating opportunities, between the sixteenth and twentieth days of the cycle; c) the failure of one male to mate with any female except on days 11 to 24, although there were some seventy opportunities on days outside this range; d) a rise in the sexual activity curve, very similar to the one presented in this report, is known to precede ovulation in monkeys. (Ball and Hartman, '35.)

Because of differences in cycle length it appears to be more exact to date the fertile period from the end rather than the beginning of the cycle. This enables us to state that the most probable dates of fertile mating all occurred from 15 to 19 days before the anticipated onset of the next menstrual period.

Using the first day of detumescence as a dividing point in the cycle, the first and second portions are examined in respect to their relations to the total cycle. It is shown that this critical date is quite variable if counted from the beginning of the cycle; that its relation to the end of the cycle, or to the following menstrual period, is much more constant, typically preceding it by 10 or 11 days.

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NEW BOOKS AND PUBLICATIONS

COLOR CHANGES OF ANIMALS IN RELATION TO NERVOUS ACTIVITY, by G. H. Parker. 1936. Size, 6 × 9 inches. Cloth. Pages ix + 74. Index. List of references. 40 illustrations. Price, $1.50. University of Pennsylvania Press, Philadelphia.

An interesting presentation of the problems of research on color changes and their control in living animals. The introduction treats of historical data and general principles. Other chapters deal with the dogfish, the killifish, neurohumors, the nervous system and chromatophores. It is an extended form of the Joseph Leidy Memorial Lecture in Science delivered at the University of Pennsylvania March 3, 1936.

NUTRITIVE AND THERAPEUTIC VALUES OF THE BANANA, A Digest of Scientific Literature. 1936. Size, 7 × 10 inches. 143 pages. Index. Research Department, United Fruit Company, Boston.

Extracts from scientific literature (292 articles) show that well-ripened bananas have excellent food values. Its qualifications include availability, palatability, digestibility, high calory content, and all vitamins except D. In certain digestive disturbances they have definite therapeutic values.

GRUNDRISS DER HISTOLOGIE UND MIKROSCOPISCHEN ANATOMIE DES MENSCHEN BIOLOGIE DER MIKROSKOPISCHEN GRÖSSENORDNUNG, by Hans Petersen. 1936. Size, 5 × 8 inches. Pages V + 169. 197 illustrations. Index. Price, RM. 6.90. Julius Springer, Berlin.

A small textbook of microscopic anatomy which begins with a discussion of protoplasm and the cell, describes types of tissues, and devotes most of its pages to the histological structure of organs and systems.

LES TROIS RACES DE L'EUROPE ET DU MONDE. Leur Génie et Leur Histoire, by Paul Buyssens. 1936. Size, 6¾ × 9 inches. 270 pages. 18 illustrations. Editions Purnal, Bruxelles.

The division of mankind into three races based on pigmentation seems, to the author of this book, unsatisfactory. He prefers another division based on combinations of physical, psychological and cultural characteristics which have been evident in the different races since the earliest traces of civilization. Humanity is separated into the Nordics developing in the north, the Mediterraneans in the intermediary zone, and the Negro in the south, the yellow peoples being included in the Negro group. Prehistoric data supporting this arrangement is offered.

VERHANDLUNGEN DER DEUTSCHEN GESELLSCHAFT FÜR KREISLAUFFORSCHUNG. IX Tagung. Edited by Eb. Koch. 1936. Size, 6¼ × 9¼ inches. Pages xx + 368. 47 illustrations. Subject and author indices. Price, RM. 15. Theodor Steinkopff, Dresden and Leipzig.

The ninth session of the society was held in conjunction with the Ärztlichen Ausschuss der Deutschen Gesellschaft für Arbeitsschutz at Bad Nauheim April 16–18, 1936. The general subject was circulatory disease in relation to social and industrial problems. Thirty-eight papers were presented.

SUPPLEMENT

AMERICAN SOCIETY OF ZOÖLOGISTS

Officers for 1936

President	W. C. Allee
Vice-President	C. W. Metz
Secretary	H. B. Goodrich
Treasurer	Walter N. Hess

Executive Committee

(In addition to the four officers)

Fernandus Payne	to serve until December, 1936
W. C. Curtis	to serve until December, 1937
Charles Zeleny	to serve until December, 1938
A. H. Sturtevant	to serve until December, 1939
Robert W. Hegner	to serve until December, 1940

Representative of the Society in the Division of Biology and Agriculture of the National Research Council

To serve until July 1, 1938	L. V. Heilbrunn

C. R. Moore, Alternate

Representatives of the Society on the Council of the American Association for the Advancement of Science

J. E. Ackert H. H. Plough

Representatives of the Society on the Council of the Union of American Biological Societies

J. T. Patterson Oscar Riddle

Representatives of the Society on the Advisory Editorial Board of "Biological Abstracts"

H. S. Pratt and G. K. Noble	*Taxonomy*
D. H. Tennent	*General Embryology*
M. H. Jacobs	*General Physiology*

Representative on the Executive Committee of the Commission for the Standardization of Biological Stains

S. I. Kornhauser

Managing Editor of the Journal of Morphology

To serve until December, 1936	C. E. McClung

Members of Editorial Board of the Journal of Morphology

To serve until December, 1936

Charles Zeleny J. F. Daniel J. S. Nicholas

To serve until December, 1937

D. H. Tennent L. C. Dunn C. G. Hartman

To serve until December, 1938

Sally Hughes-Schrader E. E. Just D. H. Wenrich

AMERICAN SOCIETY OF ZOÖLOGISTS

CONSTITUTION

Article I

NAME AND OBJECT

Section 1. The Society shall be called the "American Society of Zoölogists."

Sec. 2. The object of the Society shall be the association of workers in the field of zoölogy for the presentation and discussion of new or important facts and problems in that science and for the adoption of such measures as shall tend to the advancement of zoölogical science.

Article II

MEMBERSHIP

Section 1. The membership of the Society shall consist of two classes:

a. Associate membership open to all actively engaged in the field of zoölogy who have had training in zoölogy equivalent to that required for the doctorate degree.

b. Active membership open to those who hold the doctor's degree in zoölogy or who have done work equivalent thereto, and who, in addition to the usual requirements for this degree, have published the results of substantial zoölogical research.

Sec. 2. Election to membership in the Society shall be upon recommendation of the Executive Committee.

Sec. 3. Associate members shall not hold office or vote, but may present papers before the Society without introduction. Associate members shall receive all literature issued by the Society, including the proceedings of the Society, and shall have the same privileges as active members in journal subscriptions.

Sec. 4. Associate members may be elected to active membership in the usual way when the research requirements of active membership have been met.

Article III

OFFICERS

Section 1. The officers of the Society shall be a President, a Vice-President, a Secretary, a Treasurer, and the members at large of the Executive Committee.

Sec. 2. The Executive Committee shall consist of the President, the Vice-President, the Secretary, the Treasurer, and five members elected from the Society at large. Of these five members, one shall be elected each year to serve five years. If any member at large shall be elected to any other office, a member at large shall be elected at once to serve the remainder of his term.

Sec. 3. These officers shall be elected by ballot at the annual meeting of the Society and their official terms shall commence with the close of the annual meeting, except that the Secretary and the Treasurer shall be elected triennially and shall serve for three years.

Sec. 4. The officers named in Section 1 shall discharge the duties usually assigned to their respective offices. (See by-laws.)

Sec. 5. Vacancies in the board of officers, occurring from any cause, may be filled by election by ballot at any meeting of the Society. A vacancy in either the Secretaryship or the Treasurership occurring in the interval of the meetings of the Society may be filled, until the next annual meeting, by appointment by the Executive Committee.

Sec. 6. At the annual meeting the President shall name a nominating committee consisting of three active members. It shall be the duty of this committee to nominate officers of the Society, including the permanent representatives which the Society is asked to name to various boards and councils. This committee shall make its nominations to the Secretary at least four months before the next annual meeting. Additional nominations for any office may be made in writing to the Secretary by any five members at any time previous to balloting.

ARTICLE IV

MEETINGS OF THE SOCIETY

Section 1. Unless previously determined by the Society, the time and place of the annual meeting shall be determined by the Executive Committee. Special meetings may be called and arranged for by the Executive Committee. Notices of such meetings shall be mailed to all members of the Society at least two weeks before the date set for the meeting.

Sec. 2. Sections of the Society may be organized in any locality by not less than ten members, for the purpose of holding meetings for the presentation of scientific papers. Such sections shall have the right to elect their own officers and also associate members; provided, however, that associate membership in any section shall not confer membership in the Society.

ARTICLE V

QUORUM

Twenty-five members shall constitute a quorum of the Society and four a quorum of the Executive Committee.

ARTICLE VI

CHANGES IN THE CONSTITUTION

Amendments to this Constitution may be adopted at any meeting of the Society upon approval of two-thirds of the members voting, subject to the following conditions:

a. The proposed amendment must be in writing and signed by at least five members of the Society.

b. This signed proposal must be in the hands of the Secretary at least one month before the meeting of the Society at which it is to be considered.

c. The Secretary shall mail copies of the proposed amendment to the members of the Society at least two weeks before the meeting.

d. Members unable to attend may submit their votes in writing to the Secretary.

BY-LAWS

ARTICLE I

DUES

Section 1. The annual dues for active and associate members, unless remitted or changed by the vote of the Society, shall be two dollars.

Sec. 2. Any member in arrears of dues for two years should be dropped by the Executive Committee. Upon payment of arrearage he shall be reinstated.

ARTICLE II

PRESIDENT

Section 1. The President shall preside at scientific sessions and at the business meetings.

Sec. 2. He shall also appoint the committees prescribed by the constitution and by-laws.

ARTICLE III

VICE-PRESIDENT

Section 1. The Vice-President shall preside at sessions designated by the President.

Sec. 2. He shall also assume the duties of the President in the latter's absence.

ARTICLE IV

SECRETARY

Section 1. The Secretary shall keep the records of the Society.

Sec. 2. He shall be responsible for all arrangements for the meetings, including the appointment of local committees.

Sec. 3. Whenever the proper officers of a number of related societies shall have a conference covering matters of mutual interest to the several societies, he shall act as the delegate or representative of this Society.

Sec. 4. He shall make such purchases and employ such assistance as will, in his judgment, expedite the business of the Society, and

Sec. 5. He shall be reimbursed out of the funds of the Society for expenses incurred in attending meetings of the Society.

ARTICLE V

TREASURER

Section 1. The Treasurer shall be in charge of the funds of the Society.

Sec. 2. At the annual business meeting of the Society he shall present a statement to date of the funds of the Society.

Sec. 3. He shall make such purchases and employ such assistance as will, in his judgment, expedite the business of the Society.

ARTICLE VI

AUDITING COMMITTEE

The President shall annually appoint an auditing committee of two, who shall audit and report upon the financial record and statement of the Treasurer at the meeting for which they were appointed.

ARTICLE VII

PROGRAM RULES

In matters relating to programs for annual meetings, the following rules shall be observed:

Section 1. Titles and abstracts of papers to be presented by any method at the annual meeting must be sent to the Secretary in duplicate before the final date set by him. The length, form, and arrangement of the abstracts must comply with the requests of the Secretary in order to insure prompt publication by The Wistar Institute. The titles and abstracts of papers which do not conform to such requests or papers which are received late may be presented by title only.

Sec. 2. Titles shall be listed in groups according to subject matter, the groups to be determined by the Secretary.

Sec. 3. Whenever conditions require, the Secretary shall schedule two or more groups for the same hour and arrange the program to bring together papers on subjects of more general interest for meetings of the whole Society.

Sec. 4. Each member may have not more than fifteen minutes of the time of the Society at the annual meeting. This time may be used by him to present one or more papers personally or to introduce papers of non-members.

Sec. 5. All papers not read when called for as listed shall be placed at the end of the group list, and if not read when called for the second time, shall be read by title only.

Sec. 6. The titles of 'introduced' papers may be listed in the groups after the titles of papers to be read by members.

LIST OF FORMER OFFICERS

AMERICAN MORPHOLOGICAL SOCIETY

President	*Vice-President*	*Secretary-Treasurer*
1890—E. B. Wilson	....	J. P. McMurrich
1891—C. O. Whitman	E. L. Mark	J. P. McMurrich
1892—C. O. Whitman	H. F. Osborn	J. P. McMurrich
1893—C. O. Whitman	E. B. Wilson	J. P. McMurrich
1894—C. O. Whitman	W. B. Scott	G. H. Parker
1895—E. B. Wilson	W. B. Scott	G. H. Parker
1896—E. L. Mark	H. F. Osborn	G. H. Parker
1897—C. S. Minot	S. I. Smith	G. H. Parker
1898—H. F. Osborn	T. H. Morgan	G. H. Parker
1899—E. G. Conklin	W. M. Wheeler	Bashford Dean
1900—T. H. Morgan	H. C. Bumpus	J. S. Kingsley
1901—J. S. Kingsley	E. A. Andrews	T. H. Montgomery
1902—H. C. Bumpus	G. H. Parker	M. M. Metcalf

Additional Members of the Executive Committee

1891—E. B. Wilson
H. F. Osborn
1892—E. L. Mark
T. H. Morgan
1893—T. H. Morgan
C. B. Davenport
1894—E. A. Andrews
F. H. Herrick
1895—T. H. Morgan
S. Watase
1896—E. G. Conklin
William Patten
1897—J. S. Kingsley
Bashford Dean
1898—C. B. Davenport
F. R. Lillie
1899—J. P. McMurrich
G. H. Parker
1900—F. R. Lillie
Jacob Reighard
1901—C. F. W. McClure
C. W. Hargitt
1902—H. S. Jennings
R. G. Harrison

AMERICAN SOCIETY OF ZOÖLOGISTS

EASTERN BRANCH	*President*	CENTRAL BRANCH
G. H. Parker	1903	Jacob Reighard
E. A. Andrews	1904	C. H. Eigenmann
W. E. Castle	1905	F. R. Lillie
W. E. Castle	1906	C. C. Nutting
C. B. Davenport	1907	S. A. Forbes
W. M. Wheeler	1908	E. A. Birge
H. S. Jennings	1909	E. A. Birge
T. H. Montgomery	1910	C. E. McClung
H. V. Wilson	1911	George Lefevre
A. G. Mayer	1912	H. B. Ward
Raymond Pearl	1913	H. B. Ward

LIST OF FORMER OFFICERS

EASTERN BRANCH	*Vice-President*	CENTRAL BRANCH
Jacob Reighard	1903	H. F. Nachtrieb
W. E. Castle	1904	S. J. Holmes
William Patten	1905	William A. Locy
William Patten	1906	George Lefevre
F. H. Herrick	1907	H. B. Ward
H. S. Jennings	1908	M. F. Guyer
H. V. Wilson	1909	M. F. Guyer
H. H. Wilder	1910	H. F. Nachtrieb
H. E. Crampton	1911	R. H. Walcott
G. A. Drew	1912	C. M. Child
Alex. Petrunkevitch	1913	C. M. Child

Secretary-Treasurer

G. A. Drew	1903	Frank Smith
G. A. Drew	1904	F. R. Lillie
H. S. Pratt	1905	C. E. McClung
H. S. Pratt	1906	T. G. Lee
C. J. Herrick	1907	T. G. Lee
L. L. Woodruff	1908	T. G. Lee
L. L. Woodruff	1909	Charles Zeleny
H. W. Rand	1910	H. V. Neal
Raymond Pearl	1911	H. V. Neal
J. H. Gerould	1912	W. C. Curtis
Caswell Grave	1913	W. C. Curtis

Executive Committeemen

F. R. Lillie	Herbert Osborn
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E. A. Andrews	W. A. Locy
W. R. Coe	C. M. Child
G. A. Drew	R. H. Walcott
M. M. Metcalf	W. C. Curtis
D. H. Tennent	Oscar Riddle
R. G. Harrison	H. B. Ward
H. E. Jordan	Chauncey Juday
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AMERICAN SOCIETY OF ZOÖLOGISTS (AMALGAMATED)

	President	*Vice-President*	*Executive Committeemen*
1914.	C. E. McClung	M. F. Guyer	H. E. Jordan—1 year
1915.	W. A. Locy	W. E. Ritter	H. F. Nachtrieb—2 years
1916.	D. H. Tennent	Charles Zeleny	H. V. Wilson—3 years
1917.	M. M. Metcalf	Charles Zeleny	George Lefevre—4 years
1918.	George Lefevre	L. L. Woodruff	A. F. Shull—5 years
1919.	C. M. Child	H. H. Wilder	D. H. Tennent—5 years
1920.	Gilman A. Drew	Caswell Grave	R. P. Bigelow—5 years
1921.	Charles A. Kofoid	Aaron T. Treadwell	L. J. Cole—4 years
1922.	H. H. Wilder	Bennet M. Allen	H. V. Wilson—5 years
1923.	M. F. Guyer	Robert A. Budington	M. M. Metcalf—5 years
1924.	R. G. Harrison	R. K. Nabours	George Lefevre—5 years
1925.	C. R. Stockard	C. R. Moore	C. M. Child—5 years
1926.	S. O. Mast	W. C. Allee	G. A. Drew—5 years
1927.	S. J. Holmes	Robert Chambers, Jr.	C. A. Kofoid—5 years
1928.	Caswell Grave	M. H. Jacobs	H. H. Wilder—5 years
1929.	C. B. Davenport	Fernandus Payne	J. H. Gerould—1 year
1930.	H. V. Neal	E. E. Just	M. F. Guyer—5 years
1931.	Fernandus Payne	D. E. Minnich	R. G. Harrison—5 years
1932.	W. C. Curtis	L. V. Heilbrunn	C. R. Stockard—5 years
1933.	Charles Zeleny	J. H. Bodine	S. O. Mast—5 years
1934.	A. H. Sturtevant	H. W. Rand	S. J. Holmes—5 years
1935.	R. W. Hegner	Sewall Wright	Caswell Grave—5 years
			C. B. Davenport—4 years
			H. V. Neal—5 years

Secretary-Treasurer

1914–1918. Caswell Grave

1918–1921. W. C. Allee

	Secretary		*Treasurer*
1921–1924.	W. C. Allee	1922.	D. H. Tennent
1925–1928.	D. E. Minnich	1923–1924.	R. H. Bowen
1928–1929.	L. B. Arey	1925–1930.	L. B. Arey
1929–1930.	D. E. Minnich	1930–1933.	A. B. Dawson
1930–1933.	W. H. Cole	1933–1935.	B. H. Willier
1933–1936.	H. B. Goodrich		

Representative in the Division of Biology and Agriculture, National Research Council

M. F. Guyer 1919–1921
William Patten 1921–1924
J. T. Patterson 1924–1927
G. H. Parker 1926–1929
G. H. Parker 1919–1922
H. S. Jennings 1922–1925
L. L. Woodruff 1925–1928
E. G. Conklin 1926–1929
F. R. Lillie 1919–1923
E. G. Conklin 1923–1926
H. S. Jennings 1929–1931
L. C. Dunn 1931–1932
J. H. Bodine 1932–1935

Associate Editors of the Journal of Morphology

1921.	Gary N. Calkins	J. S. Kingsley	William Patten
1921–1922.	E. G. Conklin	M. F. Guyer	W. M. Wheeler
1921–1923.	C. A. Kofoid	F. R. Lillie	J. T. Patterson
1922–1924.	G. A. Drew	H. V. Neal	L. L. Woodruff
1923–1925.	Caswell Grave	D. H. Tennent	H. V. Wilson
1924–1926.	Helen Dean King	S. O. Mast	A. A. Schaeffer
1925–1927.	R. W. Hegner	Fernandus Payne	H. B. Ward
1926–1928.	L. J. Cole	J. H. Gerould	M. H. Jacobs
1927–1929.	W. C. Curtis	J. Percy Moore	A. F. Shull
1928–1930.	Edgar Allen	G. A. Baitsell	R. E. Coker
1929–1931.	S. R. Detwiler	R. S. Lillie	H. J. Muller
1930–1932.	W. R. Coe	E. N. Harvey	H. E. Jordan
1931–1933.	L. V. Heilbrunn	S. O. Mast	G. K. Noble
1932–1934.	D. E. Minnich	B. H. Willier	L. L. Woodruff
1933–1935.	L. R. Cleveland	F. L. Hisaw	Franz Schrader

LIST OF PLACES OF MEETING

American Morphological Society

1890—Boston
1891—Philadelphia
1892—Princeton
1893—New Haven
1894—Baltimore
1895—Philadelphia
1896—Boston
1897—Ithaca
1898—New York
1899—New Haven
1900—Baltimore
1901—Chicago
1902—Washington

Central Naturalists

1899—Chicago
1900—Chicago

Society of American Zoölogists

1901—Chicago
1902—Washington

American Society of Zoölogists

EASTERN BRANCH

1903—Philadelphia
1904—Philadelphia
1906—New York
1907—New Haven
1909—Boston
1910—Ithaca

JOINT MEETINGS

1905—Ann Arbor
1908—Baltimore
1911—Princeton
1912—Cleveland
1913—Philadelphia

CENTRAL BRANCH

1903—St. Louis
1905—(Mch.) Chicago
1907—(Mch.) Madison
1907—Chicago
1910—(Apr.) Iowa City
1910—Minneapolis
1912—(Apr.) Urbana

MEETING PLACES

1914—Philadelphia
1915—Columbus
1916—New York
1917—Minneapolis
1918—Baltimore
1919—St. Louis
1920—Chicago
1921—Toronto
1922—Boston
1923—Cincinnati
1924—Washington
1925—New Haven
1926—Philadelphia
1927—Nashville
1928—New York
1929—Des Moines
1930—Cleveland
1931—New Orleans
1932—Atlantic City
1933—Boston
1934—Pittsburgh
1935—Princeton

PROGRAM FOR THE THIRTY-FOURTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF ZOÖLOGISTS

ATLANTIC CITY, NEW JERSEY

DECEMBER 29, 30, 31, 1936

The Society meets this year in association with the American Association for the Advancement of Science in accordance with the wishes of many of the members. The symposium is held jointly with the Ecological Society of America on Experimental Populations. As authorized by vote of the Society at the last annual meeting three other special discussion sessions have been arranged. Two of these will be held jointly with other societies. They are the meeting on Genetics and Development with the Genetics Society of America, arranged by the secretaries of the two societies, and on Properties of Protoplasmic Surfaces with the Society of Cell Physiologists and arranged by Dr. Robert Chambers. The third is the program on Biological Effects of Radiations, arranged by Dr. W. C. Curtis. In all of these sessions there is a limited number of papers with the intention of allowing time for discussion.

The annual dinner for all zoölogists and friends will be held Wednesday evening and the address is to be given by Dr. Ross G. Harrison.

Especial attention is called to the Naturalists' symposium on Supra Specific Variations in Nature and in Classification which immediately follows the close of the zoölogists' meetings.

The demonstration session will be held Wednesday afternoon jointly with the Genetics Society of America. Any members of the Society who desire to present exhibits for the general scientific exhibits of the A.A.A.S. but not to be included in the zoölogists' program should communicate at once with F. C. Brown, director of exhibits, A.A.A.S., Smithsonian Institution Building, Washington, D. C.

Tickets for the zoölogists' dinner will be on sale at the registration table of the A.A.A.S.

As in former years the Society is greatly indebted to The Wistar Institute for the prompt publication and mailing of this program.

The Secretary has received no notification of special reduction of railroad rates. The existing low general rates probably preclude any extensive further reductions but members should consult their local agents in regard to possible vacation or convention rates.

PROGRAM

TUESDAY, DECEMBER 29TH

9.45 A.M. *Sect. A.* Embryology. Papers 1 to 9. Committee Room 12, Municipal Auditorium.

Sect. B. Physiology. Papers 10 to 19. Committee Room 1, Municipal Auditorium.

9.30 A.M. *Sect. C.* Biological Effects of Radiation. Discussion led by Dr. W. C. Curtis. Papers 20 to 26. Committee Room 11, Municipal Auditorium.

2.00 P.M. Joint Symposium with the Ecological Society of America on Experimental Populations. Committee Room 12, Municipal Auditorium. President W. C. Allee, presiding.

1. Experimental populations of microscopic organisms. Willis H. Johnson, Stanford University.
2. Experimental studies of insect populations. Thomas Park, Johns Hopkins University.
3. Population density as related to sex and to evolution in Cladocera. A. M. Banta, Brown University.
4. On biological principles affecting populations: human and other. Raymond Pearl, Johns Hopkins University.

9.00 P.M. Biologists' Smoker, Renaissance Room, Hotel Ambassador.

WEDNESDAY, DECEMBER 30TH

9.30 A.M. *Sect. A.* Joint Session with the Genetics Society of America on Embryology and Development. Dr. C. B. Davenport, presiding. Papers 27 to 31. Committee Room 12, Municipal Auditorium.

Sect. B. Physiology. Papers 32 to 43. Committee Room 1, Municipal Auditorium.

Sect. C. Endocrinology. Papers 44 to 53. Committee Room 11, Municipal Auditorium.

Sect. D. Animal Behavior and Physiology. Papers 54 to 63. Committee Room 14, Municipal Auditorium.

12.00 M. Annual Business Meeting of Section F, A.A.A.S., Committee Room 12, Municipal Auditorium.

12.10 P.M. Annual Business Meeting of American Society of Zoölogists, Committee Room 12, Municipal Auditorium.

2.00 P.M. Demonstrations. Papers 112 to 139. Ball Room, Municipal Auditorium. Motion picture demonstrations will be shown as scheduled in list of titles.

2.00 P.M. Joint Session with the Society of Cell Physiologists on Properties of Protoplasmic Surfaces. Committee Room 12, Municipal Auditorium. Dr. Robert Chambers, chairman.

6.45 P.M. Zoölogists' Dinner. Address by Dr. Ross G. Harrison, vice-president of Section F. Venetian Room, Hotel Ambassador. Open to zoölogists and friends.

THURSDAY, DECEMBER 31ST

9.00 A.M. *Sect. A.* Embryology. Papers 64 to 76. Committee Room 12, Municipal Auditorium.

Sect. B. Cytology. Papers 77 to 87. Committee Room 11, Municipal Auditorium.

Sect. C. Endocrinology and Physiology. Papers 88 to 97. Committee Room 1, Municipal Auditorium.

Sect. D. Morphology, Protozoölogy and Miscellaneous. Papers 98 to 111. Committee Room 14, Municipal Auditorium.

2.00 P.M. Joint Symposium with the American Society of Naturalists on Supra Specific Variations in Nature and in Classification. Committee Room 12, Municipal Auditorium.

1. From the viewpoint of zoölogy. Alfred C. Kinsey, Indiana University.
2. From the viewpoint of botany. Edgar Anderson, Missouri Botanical Gardens and Washington University.
3. From the viewpoint of paleontology. George Gaylord Simpson, American Museum of Natural History.
4. Discussion and summary. William K. Gregory, American Museum of Natural History.

The Naturalists' Dinner will be held at 6.30 P.M. in the Ocean Lounge, Hotel Ambassador. The presidential address will be given by Dr. C. E. Allen, University of Wisconsin, on Haploid and Diploid Generations.

Attention is also called to the demonstration programs of the Genetics Society of America on Tuesday morning and afternoon and to that of the American Society of Parasitologists on Wednesday morning. All demonstration sessions will be held in a room partitioned from the north end of the Ball Room on the same floor as Committee Rooms 1, 11 and 14.

LIST OF TITLES

Tuesday morning session, December 29th

This session will be held in three sections, as shown below

Section A. Embryology, 9.45 A.M.; Committee Room 12, Municipal Auditorium

1. Breeding habits and development of the Siamese fighting fish (Betta splendens) shown by motion pictures. (15 min.) (Moving pictures.) C. P. Leblond and T. C. Kramer, Yale University. (Introduced by H. B. Goodrich.)
2. The microscopical anatomy of a haploid, metamorphosed salamander (Triton taeniatus, Laur.). (15 min.) (Lantern.) Gerhard Fankhauser, Princeton University.
3. The transplantation of lateral half medullae from Amblystoma tigrinum to A. punctatum. (10 min.) (Lantern.) C. Hutchinson, Morris Biological Farm of The Wistar Institute. (Introduced by Helen Dean King.)
4. The action of synthetic male hormones upon the gonads and gonoducts of the chick embryo. (15 min.) (Lantern.) B. H. Willier, T. F. Gallagher and F. C. Koch, The Universities of Rochester and Chicago.

4A. Totipotency of regenerating mesenchyme in adult amphibians. (15 min.) (Lantern.) Oscar E. Schotté, Amherst College.

5. Ovum age and pregnancy in the guinea pig. Data from 462 artificially inseminated females. (15 min.) (Lantern.) Richard J. Blandau and William C. Young, Brown University.
6. The development of the rat egg after its implantation in a foreign cavity. (15 min.) (Lantern.) J. S. Nicholas, Yale University.
7. The parthenogenetic activation of rabbit eggs. (15 min.) (Lantern.) Gregory Pincus, Harvard University.
8. The factors controlling blastocyst growth in the rabbit. (15 min.) (Motion pictures.) Gregory Pincus and N. T. Werthessen, Harvard University. (Introduced by W. E. Castle.)
9. The development of a salamander (Amblystoma punctatum) shown by motion pictures. (15 min.) (Moving pictures.) L. S. Stone and T. C. Kramer, Yale University. (Demonstration paper introduced by Edgar Allen.)

The program in embryology is continued Wednesday morning with paper no. 27.

Section B. Physiology, 10.00 A.M.; Committee Room 1, Municipal Auditorium

10. Narcosis of frog muscle by n-butyl alcohol. (15 min.) (Lantern.) Robert W. Ramsey, The University of Rochester.
11. The injection of acetylcholine and of other aqueous solutions into isolated muscle fibers of the frog. (15 min.) Elsa M. Keil and F. J. M. Sichel, New Jersey College for Women (Rutgers University) and Howard University School of Medicine.

12. Potentials developed in contractions of the body wall of Lumbricus terrestris. (15 min.) (Lantern.) Robert Galambos and Paul Frederick Brown, Oberlin College. (Introduced by R. A. Budington.)
13. Excitation of the giant fiber system in the squid, Loligo pealei. (10 min.) (Lantern.) C. Ladd Prosser, John Z. Young and A. H. Chambers, Marine Biological Laboratory, Woods Hole.
14. Are there antidromic responses in the melanophore system? (15 min.) (Lantern.) G. H. Parker, Harvard University.
15. Elimination of neutral red by the frog's kidney. (8 min.) (Lantern.) Robert Chambers and Rudolf T. Kempton, Washington Square College, New York University and Princeton University.
16. Excretory products of the Mediterranean flour moth larva (Ephestia kühniella Zeller). (15 min.) (Lantern.) Nellie M. Payne, University of Minnesota.
17. Nutrition studies with the cockroach, Blattella germanica. (12 min.) (Lantern.) R. M. Melampy and L. A. Maynard, Cornell University. (Introduced by Wm. H. Gates.)
18. The cleavage of Arbacia eggs under hydrostatic compression. (7 min.) (Lantern.) Douglas Marsland, Washington Square College, New York University. (Introduced by Robert Chambers.)
19. Effect of age on Arbacia egg membrane. (10 min.) (Lantern.) A. J. Goldforb, College of the City of New York.

The program in physiology is continued Wednesday morning in two sections beginning with papers nos. 32 and 54.

Section C. Special meeting on biological effects of radiation, 9.30 A.M.; Committee Room 11, Municipal Auditorium

Discussion led by W. C. Curtis

20. The isolation of desired qualities of radiant energy for biological experimentation. (15 min.) (Lantern.) Loyd A. Jones and C. Tuttle, Kodak Research Laboratories. (Introduced by the Secretary.)
21. Ultraviolet radiations as experimental means for the investigation of protoplasmic behavior. (15 min.) (Lantern.) E. E. Just, Howard University.
22. Further studies on the effect of radiation on protoplasm. (15 min.) (Lantern.) L. V. Heilbrunn, University of Pennsylvania.
23. Ultraviolet irradiation of microorganisms and some interpretations. (20 min.) (Lantern.) B. M. Duggar and Alexander Hollaender, University of Wisconsin. (Introduced by the Secretary.)
24. A respiratory stimulating factor. (15 min.) (Lantern.) John C. Fardon, Robert J. Norris and John R. Loofbourow, Institutum Divi Thomae. (Introduced by W. C. Curtis.)
25. Intensity discrimination and response to flicker. (15 min.) W. J. Crozier, Harvard University.
26. Response to light in Peranema trichophorum with special reference to dark-adaptation and light-adaptation. (15 min.) (Lantern.) S. O. Mast and Landrum B. Shettles, Johns Hopkins University.

Tuesday afternoon session, December 29th, 2.00 P.M.; Committee Room 12, Municipal Auditorium

Joint meeting with the Ecological Society of America

Symposium: EXPERIMENTAL POPULATIONS

President W. C. Allee, presiding

1. Experimental populations of microscopic organisms. Willis H. Johnson, Stanford University.
2. Experimental studies of insect populations. Thomas Park, Johns Hopkins University.
3. Population density as related to sex and to evolution in Cladocera. A. M. Banta, Brown University.
4. On biological principles affecting populations: human and other. Raymond Pearl, John Hopkins University.

Tuesday evening session, *Biologists' Smoker,* 9.00 P.M.; Renaissance Room, Hotel Ambassador

Wednesday morning session, December 30th

This session will be held in four sections, as shown below

Section A. Joint meeting with the Genetics Society of America and with the American Society of Naturalists, 9.30 A.M.; Committee Room 12, Municipal Auditorium

GENETICS AND DEVELOPMENT

C. B. Davenport, presiding

27. Phenomena observed during the embryogenetic process and their significance for a theory of the physiology of development and of heredity. E. E. Just, Howard University.
28. The genetic basis of developmental relationships. Edmund W. Sinnott, Columbia University.
29. Genetics and development of Drosophila as studied by transplantation. G. W. Beadle, Harvard University.
30. The influence of nuclear factors in hybrid development analyzed by transplantations. Victor C. Twitty, Stanford University.
31. An analysis of certain phases of plumage pattern. L. V. Domm, The University of Chicago.

The program in embryology is continued as a demonstration program Wednesday afternoon with paper 112 and for papers read Thursday morning with paper no. 64.

Section B. Physiology, 9.30 A.M.; Committee Room 1, Municipal Auditorium

32. Effects of various ions on the rhythmic movements of embryos (Acrididae, Orthoptera). (10 min.) (Lantern.) Vincent Thompson, State University of Iowa. (Introduced by J. H. Bodine.)
33. The effect of certain stimulating and inhibiting substances on respiration of active and blocked eggs and isolated embryos. (5 min.) (Lantern.) J. H. Bodine and E. J. Boell, State University of Iowa.
34. Temperature studies on the egg and young of the smooth green snake. (10 min.) (Lantern.) M. X. Zarrow and C. M. Pomerat, Clark University and Harvard University. (Introduced by A. B. Dawson.)
35. A study of the variation in the metabolic rate of man and rats. (8 min.) (Lantern.) N. T. Werthessen, Harvard University. (Introduced by T. J. B. Stier.)
36. Red cell fragility in guinea pigs after splenectomy. (15 min.) Albert S. Gordon, William Kleinberg and Eric Ponder, Washington Square College, New York University, and The Biological Laboratory, Cold Spring Harbor.
37. Physiological adaptations of animals to high altitudes. (15 min.) (Lantern.) F. G. Hall, Duke University.
38. Total dissociation of horse hemoglobin. (15 min.) Jacinto Steinhardt, University of Upsala and Cambridge University. (Introduced by Selig Hecht.)
39. Similarity of the effects of heavy water and low temperature on the electrical potential of frog skin. (10 min.) (Lantern.) T. Cunliffe Barnes, Yale University.
40. On the mechanism of the ultraviolet absorption in living cells. (10 min.) (Lantern.) B. J. Luyet and P. M. Gehenio, St. Louis University.
41. Action of glycosides on hexose oxidation by luminous bacteria. (10 min.) (Lantern.) Frank H. Johnson, Princeton University. (Introduced by E. Newton Harvey.)
42. The sulphydryl distribution in Amoeba proteus as shown by the nitroprusside reaction. (15 min.) (Lantern.) H. W. Chalkley, National Institute of Health.
43. Pharmacological studies on extracts of periodical cicadas and of grasshoppers. (12 min.) (Lantern.) David I. Macht, Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Introduced by George R. LaRue.)

Section C. Endocrinology, 9.30 A.M.; Committee Room 11, Municipal Auditorium

44. Effect of castration and oestrin treatment on relative amounts of F.S.H. and L.H. in the hypophysis. (15 min.) (Lantern.) Samuel L. Leonard, Union College.
45. Experimental studies of the anterior pituitary. IV. The replacement capacity and non-cyclic behavior of homoplastic anterior pituitary grafts in the guinea pig. (15 min.) (Lantern.) M. Schweizer, H. A. Charipper and H. O. Haterius, Washington Square College, New York University, and Ohio State University.

46. Experimental ovulation and pseudopregnancy in the hypophysectomized rabbit after partial gonadal regression. (15 min.) (Lantern.) Mark A. Foster, Harvard University.
47. Follicular growth and ovulation under experimental conditions in the guinea pig. (15 min.) (Lantern.) Edward W. Dempsey and William C. Young, Brown University. (Introduced by A. D. Mead.)
48. Accentuation of the growth effect of theelin on genital tissues by arrest of mitosis with colchecin. (15 min.) (Lantern.) Edgar Allen, G. M. Smith and W. U. Gardner, Yale University.
49. Interstitial cell hypertrophy in the testes of mice receiving estrogenic hormones. (15 min.) (Lantern.) W. U. Gardner, Yale University. (Introduced by Ross G. Harrison.)
50. Pituitary-like effects of yeast extracts. (15 min.) (Lantern.) Frederick L. Hisaw, R. O. Greep and H. L. Fevold, Harvard University.
51. The induction of brooding behavior in the jewel fish. (15 min.) (Lantern.) G. K. Noble, K. F. Kumpf and V. N. Billings, American Museum of Natural History.
52. Reciprocal weight changes in liver and testis of pigeons during reproduction. (8 min.) (Lantern.) J. P. Schooley and Oscar Riddle, Carnegie Institution.
53. Sex hormones and serum calcium. (7 min.) (Lantern). Oscar Riddle and Louis B. Dotti, Carnegie Institution.

The program in endocrinology is continued as a demonstration program Wednesday afternoon with paper no. 121 and Thursday morning with paper no. 88.

Section D. Animal Behavior and Physiology, 9.30 A.M.; Committee Room 14, Municipal Auditorium

54. The place of origin and manipulation of cnidoblasts by Chlorohydra viridissima. (15 min.) (Opaque projection.) William A. Kepner, R. A. Goodman and A. Ingles, University of Virginia.
55. Reactions of Limulus to illuminated fields of different area and flicker frequency. (10 min.) (Lantern.) Ernst Wolf, Harvard University. (Introduced by Leigh Hoadley.)
56. The relative sensitivity to light of ommatidia in various parts of the compound eye of the drone fly, Eristalis tenax. (15 min.) (Lantern.) William L. Dolley, Jr., and Mary Alice Hartwell, University of Buffalo.
57. Responses to light in the earthworm, Pheretima sp. (15 min.) (Lantern.) Charles D. Howell, Johns Hopkins University. (Introduced by W. G. Lynn.)
58. Nocturnal activity rhythm in bats under constant external conditions. (10 min.) (Lantern.) J. H. Welsh and D. R. Griffin, Harvard University.
59. Effects of various periods of starvation on growth, reproduction, heart rate and longevity in Cladocera. (15 min.) (Lantern.) Lester Ingle, Brown University and The University of Chicago. (Introduced by A. M. Banta.)
60. Interpretations of data on growth and reproduction (Daphnia longispina). (15 min.) (Lantern.) Thelma R. Wood and A. M. Banta, Brown University and Carnegie Institution of Washington.
61. Water absorbing properties of oligochaete tissues. (10 min.) Mary Elizabeth Kopenhaver, Lake Forest College. (Introduced by J. Wm. Buchanan.)

62. Some effects of strychnine on reconstitution in Euplanaria dorotocephala. (15 min.) Faith Stone Miller, The University of Chicago. (Introduced by C. M. Child.)
63. Studies on heteroplastic transplantation in Triclads. (15 min.) James A. Miller, Ohio University. (Introduced by Carl R. Moore.)

The program on physiology is continued Thursday morning with paper no. 88.

Wednesday noon *business session of Section F, A.A.A.S.*, December 30, 12.00 M.; Committee Room 12, Municipal Auditorium, followed immediately by *business session of the American Society of Zoölogists*, December 30, 12.10 P.M.; Committee Room 12, Municipal Auditorium.

Wednesday afternoon session, December 30, 2.00 P.M.; Ball Room, Municipal Auditorium

Joint session with Genetics Society of America

DEMONSTRATIONS

Embryology

112. Intergeneric graft hybrids of Ecteinascidia and Perophora (Ascidians). H. H. Plough, Amherst College.
113. Regeneration and differentiation in Crustacea. Hugh H. Darby, College of Physicians and Surgeons, Columbia University.
114. Incompatibility in species and local races of Amblystoma during parabiosis or following transplantation of tissues. R. R. Humphrey and R. K. Burns, Jr., University of Buffalo School of Medicine and University of Rochester School of Medicine and Dentistry.
115. The growth of an urodele larva as illustrated by transformed coordinates. Oscar W. Richards and G. A. Riley, Yale University.
116. Microscopical preparations of bone marrow of turkey, showing the relation of lymphoid nodules to blood production. H. E. Jordan, University of Virginia.
117. Development of the transplanted inner ear rudiment of the rabbit embryo. A. J. Waterman, Williams College.
118. A cyclops lamb. A. M. Reese, West Virginia University.

Physiology

119. Stimulation of the hypophysis and genital glands in the duck by electric light. Effect of thyroidectomy on the testis and liver. Jacques Benoit, Yale University School of Medicine. (Introduced by Edgar Allen.)
120. Phenomena of regeneration and growth, injury and recovery, contraction and retraction of striated muscle fibers. Carl C. Speidel, University of Virginia Medical School. To be shown at 3.30 P.M., Committee Room 11.

Endocrinology

121. The effect of pituitary hebin upon the reproductive system of the chick embryo. L. V. Domm and E. A. Dennis, The University of Chicago and American University.

122. Feather sequences grown from single follicles in the saddle of the hybrid capon and female hormone modifications. Mary Juhn, The University of Chicago.
123. Further experiments on hormonal control of the seasonal plumage characters in Pyromelana franciscana (African weaver finch). Emil Witschi, State University of Iowa.
124. The histology of precociously activated thyroids of the tadpole. William Etkin, City College and American Museum of Natural History.
125. Specificity of the pituitary gonadotropic factor in amphibians as demonstrated by implants obtained from fishes. Charles W. Creaser and Aubrey Gorbman, Wayne University.
126. Vaginal changes, heat and the time of ovulation in the guinea pig. William C. Young and Edward W. Dempsey, Brown University.

Cytology

127. Cytology of abnormal growths produced by x-ray irradiation of grasshopper eggs. E. Eleanor Carothers, University of Iowa.
128. Isolation of interkinetic chromosome pairs from germinal vesicles of the ovarian eggs of Rana pipiens. William R. Duryee, Columbia University.
129. The non-homogeneous character of the nuclear membrane. William R. Duryee, Columbia University.
130. New criteria for distinguishing living and dead cells. B. J. Luyet and P. M. Gehenio, St. Louis University.
131. Cytology and development of flame cells in the redial generation of a trematode. Charles H. Willey, New York University.
132. A study of the bacterial and alleged mitochondrial content of the cells of the clover nodule. E. DeWitt Miller, University of Virginia.
133. Cupulae on the lateral-line organs of Fundulus heteroclitus. Martha Denny, Connecticut College. (Introduced by Pauline H. Dederer.)
134. The effect of chloretone on tissue preceding fixation. Robert T. Hance, University of Pittsburgh.
135. An improved and inexpensive microtome knife. Robert T. Hance, University of Pittsburgh.
136. How things grow. W. J. Baumgartner, University of Kansas. To be shown at 4.00 P.M. in Committee Room 11.

Protozoölogy

137. Cultures of living Chaos chaos Lin. A. A. Schaeffer, Temple University.

Parasitology

138. Studies on Clinostomum. V. The cyst of the yellow grub of fish (C. marginatum). George W. Hunter, 3rd and H. Clark Dalton, Wesleyan University.
139. The larval form of Foleyella americana Walton, 1929. A. C. Walton, Knox College.

Wednesday afternoon session, December 30, 2.00 P.M.; Committee Room 12, Municipal Auditorium

Joint session with Society of Cell Physiologists

PROPERTIES OF PROTOPLASMIC SURFACES

Chairman, Robert Chambers

1. Electrical impedance of cell surfaces. K. C. Cole, Columbia University Medical School.
2. Physical properties of the red cell membrane. Eric Ponder, Biological Laboratory, Cold Spring Harbor.
3. Penetration of oils into Echinoderm ova. Robert Chambers and M. J. Kopac, Washington Square College, New York University.
4. The surface precipitation reaction. L. V. Heilbrunn, University of Pennsylvania.
5. Permeability studies on the plasma membrane. M. H. Jacobs, University of Pennsylvania.

Wednesday evening session, *Zoölogists' Dinner*, December 30th, 6.45 P.M.; Venetian Room, Hotel Ambassador

Address by vice-president of Zoölogical Section (F), Ross G. Harrison, Yale University.

All zoölogists and friends are invited.

Thursday morning session, December 31st
This session will be held in four sections as shown below.

Section A. Embryology, 9.00 A.M.; Committee Room 12, Municipal Auditorium

64. Transplantation of the polar lobe in Sabellaria vulgaris. (7½ min.) (Lantern). Alex B. Novikoff, Columbia University. (Introduced by L. G. Barth.)
65. Microcautery of posterior pole-plasm and germ-cell determinants in Drosophila melanogaster. (5 min.) (Opaque projection.) Joseph W. Jailer, Washington Square College, New York University. (Introduced by R. B. Howland.)
66. The development and metamorphosis of the ascidian Phallusia nigra. (15 min.) (Motion picture.) Oscar W. Richards, Yale University.
67. Regeneration and differentiation in Crustacea. (15 min.) (Lantern.) Hugh H. Darby, College of Physicians and Surgeons, Columbia University.
68. Growth of the chick embryo. (15 min.) (Lantern.) T. C. Byerly, W. G. Helsal and J. P. Quinn, Bureau of Animal Industry.
69. X-ray studies in the genetics and morphogenesis of Drosophila melanogaster. (7 min.) (Lantern.) C. P. Haskins and E. V. Enzmann, Harvard University. (Introduced by T. J. B. Stier.)
70. Effects of roentgen radiation on embryonic organization and regulation in Melanoplus differentialis (Orthoptera). (7 min.) (Lantern.) Titus Carr Evans, University of Iowa and A. and M. College of Texas.
71. Use of roentgen radiation in analysis of metabolism in eggs of Melanoplus differentialis. (8 min.) (Lantern.) Titus Carr Evans, University of Iowa and A. and M. College of Texas.

72. The susceptibility of Fundulus heteroclitus embryos to x-radiation. (7½ min.) (Lantern.) Archie N. Solberg, Columbia University. (Introduced by L. G. Barth.)
73. X-radiation and thyroid-induced metamorphosis in Anuran larvae. (15 min.) (Lantern.) William O. Puckett, Princeton University. (Introduced by C. F. W. McClure.)
74. Paper withdrawn; to be read by title.
75. The ovarian cycle of the viviparous teleost, Skiffia bilineata, a member of the family Goodeidae. (15 min.) (Lantern.) Guillermo Mendoza, Northwestern University. (Introduced by D. L. Turner.)
76. The reproductive cycle in the males of some cave bats. (12 min.) (Lantern.) Roland E. Miller, University of Missouri. (Introduced by Mary J. Guthrie.)

Section B. Cytology, 9.00 A.M.; Committee Room 11, Municipal Auditorium

77. Experimental modification of chromosome behavior during reduction divisions in the egg of Nassa (Ilyanassa). (7 min.) (Lantern.) G. B. Moment, Goucher College.
78. The frequency of reciprocal translocations in irradiated germ cells of Circotettix verruculatus (Orthoptera). (15 min.) (Lantern.) Edwin R. Helwig, University of Pennsylvania.
79. Multiple chromosome complexes in the metamorphosis of Culex pipiens. (8 min.) (Lantern.) C. A. Berger, Johns Hopkins University. (Introduced by C. W. Metz.)
80. Studies on salivary gland chromosomes in diptera. (7 min.) (Lantern.) C. W. Metz, Carnegie Institution of Washington.
81. The dependence of nuclear colloidal organization upon the cation ratios of a Ringer medium. (15 min.) (Lantern.) William R. Duryee, Columbia University.
82. An atypical meiotic division in the parasitic wasp Nemeritis. (10 min.) (Lantern.) B. R. Speicher, Columbia University. (Introduced by P. W. Whiting.)
83. The physiological properties of the rabbit oocyte and its sheaths. (15 min.) (Lantern.) D. S. de Renyi and D. P. Murphy, University of Pennsylvania. (Introduced by S. A. Matthews.)
84. Polar protoplasmic behavior during cell division. (15 min.) (Lantern.) W. J. Baumgartner, University of Kansas.
85. Distribution of minerals in the cells of some human tissues. (15 min.) (Lantern.) Gordon H. Scott, Washington University School of Medicine. (Introduced by E. V. Cowdry.)
86. The cytological changes produced in the basophilic cells of the hypophysis by various treatments of the testes. (15 min.) (Lantern.) Arthur J. Gatz, Carleton College. (Introduced by D. E. Minnich.)
87. A study of the bacterial and alleged mitochondrial content of the cells of the clover nodule. (15 min.) (Lantern.) E. DeWitt Miller, University of Virginia.

Section C. Endocrinology and Physiology, 9.00 A.M.; Committee Room 1, Municipal Auditorium

88. The physical basis of accelerated barb formation by female hormone and thyroxin in certain feather follicles. (15 min.) (Lantern.) Richard M. Fraps, The University of Chicago. (Introduced by Frank R. Lillie.)
89. Variation in successively regenerated feathers from single follicles of hybrid fowl and their reaction to female hormone. (F_1, Barred Rock ♂—Brown Leghorn ♀, and reciprocal mating.) (15 min.) (Lantern.) Mary Juhn, The University of Chicago.
90. Further experiments on the thyrotropic field around the immature tadpole's pituitary. (12 min.) (Lantern.) William Etkin, City College and American Museum of Natural History.
91. Relation of the adrenal cortex to hair growth in the white rat as shown by removal and transplantation experiments. (10 min.) (Lantern.) Earl O. Butcher, Hamilton College.
92. Adrenal insufficiency. A study in general physiology. I. Blood osmotic pressure. (10 min.) (Lantern.) E. W. Blanchard, Bryn Mawr College and Biological Laboratory, Cold Spring Harbor.
93. The histology of the mammary glands of adrenalectomized lactating rats. (10 min.) (Lantern.) Irving Levenstein, Washington Square College, New York University. (Introduced by Harry A. Charipper.)
94. On the effects of hormones of spermatic fluid upon the oyster. (15 min.) (Lantern.) T. C. Nelson, Rutgers University.
95. Naturation duration of larval life in Phallusia nigra. (15 min.) (Lantern.) Caswell Grave, Washington University.
96. Cultures of some marine invertebrate tissues. (10 min.) (Lantern.) Ralph Buchsbaum, The University of Chicago.
97. Cell size and growth in mosquito larvae. (10 min.) (Lantern.) William Trager, Rockefeller Institute for Medical Research.

Section D. Morphology, Protozoölogy and Miscellaneous, 9.00 A.M.; Committee Room 14, Municipal Auditorium

98. The nervous system of Colichoglossus kowalevskyi. (10 min.) (Lantern.) Walter N. Hess, Hamilton College.
99. Observations on the enteric nerve plexus of the earthworm, Lumbricus terrestris. (8 min.) (Lantern.) Charles G. Rogers, Oberlin College.
100. Branchial reduplications found in Amblystoma. (15 min.) (Lantern.) William W. Ballard, Dartmouth College. (Introduced by W. B. Unger.)
101. The relation of germ cell degeneration to modifications in the testicular structure of Plethodontid salamanders. (12 min.) (Lantern.) J. Wendell Burger, Princeton University. (Introduced by E. G. Butler.)
102. The effect of variations in the length of the interendomictic interval on the lengths of subsequent intervals in Paramecium aurelia. (7 min.) (Charts.) T. M. Sonneborn, Johns Hopkins University.
103. The relation between time since the last endomixis and the tendency for endomixis to recur in Paramecium aurelia. (8 min.) (Charts.) T. M. Sonneborn, Johns Hopkins University.

104. The relation between locomotion and temperature in the marine amoeba, Flabellula mira Schaeffer, with special reference to adaptation to temperature. (15 min.) (Lantern.) D. L. Hopkins, Duke University.
105. Chaos chaos Lin., the rare giant ameba first seen by Roesel in 1755. (15 min.) (Lantern and moving pictures.) A. A. Schaeffer, Temple University.
106. Studies on the Trichodina (Protozoa, Ciliata) from the digestive tract of the sea-cucumber, Thyone briareus Lesueur. (15 min.) (Lantern.) Laura N. Hunter, University of Pennsylvania. (Introduced by D. H. Wenrich.)
107. Adolescariae from Agriolimax agrestis. (12 min.) (Lantern.) Bruce D. Reynolds, University of Virginia.
108. Sex organs and reproduction in Campeloma rufum, a fresh water snail. (10 min.) Norman T. Mattox, University of Illinois. (Introduced by H. J. Van Cleave.)
109. A new order of dibranchiate cephalopods. (15 min.) (Lantern.) Grace E. Pickford, Yale University.
110. A study of relative growth in Notonecta undulata. (15 min.) (Lantern.) Leonard B. Clark and A. H. Hersh, Union College and Western Reserve University.
111. Science in fish culture. I. With special reference to its needs. (15 min.) H. P. K. Agersborg, National Park Service.

PAPERS PRESENTED BY TITLE

Embryology

140. The role of l-aspartic acid in the developmental growth of Obelia geniculata. F. S. Hammett and Hans Schlumberger, Marine Experimental Station of the Lankenau Hospital Research Institute, North Truro, Mass.
141. The surface layer of the germinal vesicle of the egg of Sabellaria vulgaris. A. J. Waterman, Williams College.
142. Protandry in the Virginia oyster. W. R. Coe, Yale University.
143. Susceptibility of Bruchid (Coleoptera) eggs to KCN. Alfred Brauer, University of Kentucky.
144. Reproductive cycles and superfoetation in the Poeciliidae, a family of tropical and sub-tropical ovo-viviparous fishes. C. L. Turner, Northwestern University.
145. Replacement of the anterior limb by a grafted medulla in the embryo of the anuran Discoglossus, and its relations with the law of bilateral symmetry of neurons. Raoul M. May and Alice Frank, University of Paris.
146. Maturity and secondary sex characters in adult parabiotic twins of salamanders. Emil Witschi, State University of Iowa.
147. Differentiation in vitro of the isolated mesentodermal layer of the chick blastoderm. Dorothea Rudnick, University of Rochester.
148. The glycogenic function of hepatic tissue in normal livers and in chorio-allantoic grafts. A. J. Dalton, Western Reserve University.
149. Development of the oviducts of the red-tailed hawk, Buteo borealis borealis (Gmelin). Allan J. Stanley, State University of Iowa. (Introduced by Emil Witschi.)

150. Spermatogenic activity in first year (juvenile) sparrows, Passer domesticus. Gardner M. Riley, State University of Iowa. (Introduced by Emil Witschi.)
151. The differentiation in the rat of gonad primordia grafted to gonadectomized hosts. Adrian Buyse, University of Rochester.

Physiology

152. The activation and depression of yeast respiration by radiations. John C. Fardon, Robert J. Norris and Sister M. Veronita Ruddy, O.P., Institutum Divi Thomae. (Introduced by W. C. Curtis.)
153. The prolongation of life in Arbacia egg cells. Victor Schechter, College of the City of New York.
154. Temperature dependence of metabolic change on fertilization in Chaetopterus eggs. Herbert Shapiro, Princeton University.
155. A study of the process of fission in the naid, Pristina longiseta. C. D. Van Cleave, University of Pennsylvania.
156. Further experiments on posterior regeneration and the nerve cord in the earthworm Eisenia. P. L. Bailey, Jr., Marine Biological Laboratory, Woods Hole, and the College of the City of New York. (Introduced by R. C. McCardle.)
157. Production of accessory tails in the earthworm by posterior bisection. P. L. Bailey, Jr., Marine Biological Laboratory, Woods Hole, and the College of the City of New York. (Introduced by R. C. McCardle.)
158. Electrical inhibition in the heart of the oyster. Edward M. Walzl, Johns Hopkins University.
159. Effect of calcium and potassium on the threshold of excitation of the heart of the oyster. Edward M. Walzl, Johns Hopkins University.
160. Seasonal weight and total water content of the mound-building ant, Formica exsectoides, Forel. William A. Dreyer, University of Cincinnati.
161. The repugnatorial glands of Onchidium. Leslie B. Arey, Northwestern University Medical School.
162. Electrical variations in the heart of the adult grasshopper (Melanoplus differentialis). Theodore Louis Jahn, Frederick Crescitelli and Aubrey Bryant Taylor, State University of Iowa.
163. Resistance and capacitance measurements on grasshopper egg membranes over a wide frequency range. Kenneth S. Cole and Theodore Louis Jahn, College of Physicians and Surgeons, Columbia University, State University of Iowa, and Biological Laboratory, Cold Spring Harbor.
164. A biological test of the inverse square law of radiant energy as applied to roentgen radiation. H. Dabney Kerr and Titus C. Evans, State University of Iowa and A. and M. College of Texas.
165. Ultraviolet irradiation of grasshopper embryos (Melanoplus differentialis). O. Malcolm Ray, State University of Iowa. (Introduced by H. W. Beams.)
166. Resistance of grasshopper embryos to hypertonic solutions. Arthur Louis Schipper, State University of Iowa. (Introduced by H. W. Beams.)
167. Effect of dinitrophenols on respiration of grasshopper embryos. Joseph Hall Bodine and Edgar J. Boell, State University of Iowa.
168. Effect of temperature upon the 'block-mechanism' (diapause) of the grasshopper egg, M. differentialis. J. H. Bodine, State University of Iowa.

169. Effect of cyanide on respiration of Melanoplus differentialis eggs. Wilbur Robbie, State University of Iowa. (Introduced by J. H. Bodine.)
170. The effects of hypo- and hypertonic solutions on the tissues of fish. R. T. Young, Scripps Institute.
171. The effect of balanced and unbalanced solutions, of pH and distilled water on fish. R. T. Young, Scripps Institute.
172. The rate of adaptation of Fundulus in sea water to black and to white backgrounds at different temperatures. William H. Cole and K. F. Schaeffer, Rutgers University.
173. An independent response to light on the part of the scale melanophores of Tautoga onitis (L.). Dietrich C. Smith, University of Tennessee College of Medicine.
174. The effect of light on sexual activity in the lizard, Anolis carolinensis. H. J. Clausen and E. G. Poris, American Museum of Natural History.
175. The pineal of the lizard Anolis carolinensis, a photoreceptor as revealed by oxygen consumption studies. H. J. Clausen and B. Mofshin, American Museum of Natural History.
176. Cheyne-Stokes respiration; a study in the control of rhythm. F. H. Pike, Columbia University.
177. Effect of splenectomy on thyroxine tolerance. Albert S. Gordon and William Kleinberg, Washington Square College, New York University.
178. Longevity of erythrocyte and reticulocyte in normal and splenectomized guinea pigs. Albert S. Gordon and William Kleinberg, Washington Square College, New York University.
179. A brain disorder in young chicks fed on a diet treated with ferric chloride for destruction of vitamin E. F. B. Adamstone, University of Illinois.
180. Synthesis of vitamin C. C. P. Leblond, Yale University. (Introduced by Edgar Allen.)
181. Light in its relation to activity and estrus rhythms in the albino rat. Ludvig G. Browman, The University of Chicago.

Endocrinology

182. The chromatophorotropic hormones. A. A. Abramowitz, Harvard University (Introduced by F. L. Hisaw.)
183. A comparative study of water metabolism in amphibians injected with pituitrin. F. R. Steggerda, University of Illinois.
184. Modifications in the thyroids of the English sparrow following injection of thyreotropic hormone. Dorothea Starbuck Miller, University of Iowa. (Introduced by Paul L. Risley.)
185. Changes in blood fats following bilateral adrenalectomy. E. H. Yeakel, Bryn Mawr College and Biological Laboratory, Cold Spring Harbor. (Introduced by E. W. Blanchard.)
186. The effect of thyroidectomy upon lactation in the rat and guinea pig. W. O. Nelson and C. E. Tobin, Wayne and Yale Universities.
187. The effect of various sex hormones on the testes of hypophysectomized rats. W. O. Nelson, Wayne and Yale Universities.
188. The effect of oestrone and of pituitary extracts on the mammary glands of the rat. W. O. Nelson and C. E. Tobin, Wayne and Yale Universities.

189. Indications of a certain constancy in the character of heat in individual guinea pigs. William C. Young, Edward W. Dempsey, John L. Boling and Carl W. Hagquist, Brown University.
190. The measurement of sex drive in the female guinea pig. William C. Young, Edward W. Dempsey, Carl W. Hagquist and John L. Boling, Brown University.
191. Homotransplantation of prepubertal rat testes to the anterior chamber of the eye. Clarence D. Turner, University of Missouri and University of Georgia. (Introduced by Mary J. Guthrie.)
192. The sexual behavior and secondary sexual characters of gonadectomized fish. G. K. Noble and K. F. Kumpf, American Museum of Natural History.

Cytology

193. Ultracentrifuging and the first cleavage plane in Ascaris suum. H. W. Beams and R. L. King, State University of Iowa.
194. The chromosomes and chromatoid bodies of the western crayfish, Potamobius trowbridgei Stimpson. Nathan Fasten, Oregon State College.
195. The centriole in the parthenogenetic and fertilized eggs of Habrobracon juglandis. Elias M. Spotkov, Washington Square College, New York University. (Introduced by Alfred F. Huettner.)
196. The effect of anterior pituitary extract on the formation of meiotic spindles in oocytes. Katharine R. Jeffers and Mary J. Guthrie, University of Missouri.
197. The relationship of the Golgi material to secretion in the basophilic cells of the hypophysis in the albino rat. Arthur J. Gatz, Carleton College. (Introduced by D. E. Minnich.)

Morphology

198. Two types of respiration in Onchidium. Leslie B. Arey, Northwestern University Medical School.
199. Placoid scale types and their distribution in Squalus acanthias. Leonard P. Sayles, College of the City of New York and Marine Biological Laboratory.
200. The problem of specificity of form in cuticular scales, and of specificity in hair-shaft structure associations among the Mammalia. Leon Augustus Hausman, New Jersey College for Women.
201. The occurrence of unilateral and bilateral ovaries in raptorial birds. L. V. Domm, The University of Chicago.
202. Weights and linear measurements of the digestive system of the adult cat. Homer B. Latimer, University of Kansas.
203. A quantitative study of the skeleton of the mourning dove, Zenaidura macrura carolinensis. Homer B. Latimer and C. Willet Asling, University of Kansas.

Protozoölogy

204. The influence of certain auxins on growth in Protozoa. Alfred M. Elliott, Minnesota State Teachers College.
205. The relation between the food supply and the rate of locomotion in the marine amoeba, Flabellula mira Schaeffer. D. L. Hopkins, Duke University.

206. The removal of toxic substances from freshly prepared artificial sea water by means of sodium silicate. D. L. Hopkins, Duke University.
207. The nature and significance of the Golgi material in the marine amoeba, Flabellula mira Schaeffer. D. L. Hopkins, Duke University.
208. Growth of Euglena stellata in darkness. R. P. Hall, New York University.
209. Effects of $MnCl_2$ on growth of certain Protozoa. R. P. Hall, New York University.
210. Growth of two species of Astasia in relation to pH of the medium. H. W. Schoenborn, New York University. (Introduced by R. P. Hall.)
211. The effects of magnesium and calcium on growth in Chilomonas paramecium. S. O. Mast and D. M. Pace, Johns Hopkins University.
212. The effect of ultracentrifuging on Paramecium, with special reference to recovery and macronuclear reorganization. R. L. King and H. W. Beams, State University of Iowa.
213. Cytological notes on the Chlamydodontidae. Mary Stuart MacDougall, Agnes Scott College.
214. The vacuolar system of the Euglenida. Libbie H. Hyman, American Museum of Natural History.
215. A study of Euglena rubra (Hardy, '11). Leland P. Johnson, Iowa Lakeside Laboratory and State University of Iowa. (Introduced by R. L. King.)
216. A colorless euglenoid flagellate, Khawkinea halli, n. gen., n. sp. Theodore Louis Jahn and Wendell R. McKibben, State University of Iowa and Iowa Lakeside Laboratory.
217. Licnophora lyngbycola, n. sp. E. Fauré-Fremiet, College de France.
218. On the occurrence of Tillina in Minnesota, with a description of some new morphological structures. John P. Turner, University of Minnesota.

Ecology

219. Temperature changes in the Okoboji lakes, summer, 1936. Theodore Louis Jahn and Aubrey Bryant Taylor, Iowa Lakeside Laboratory and State University of Iowa.
220. Dissolved oxygen content of the Okoboji lakes, summer, 1936. George E. Jay, Jr., Iowa Lakeside Laboratory and State University of Iowa. (Introduced by R. L. King.)
221. A preliminary note on the ecology of the psammon of some Wisconsin lakes. Robert W. Pennak, University of Wisconsin. (Introduced by C. Juday.)
222. Science in fish culture. II. With special reference to bioecological hatcheries or rearing stations. H. P. K. Agersborg, National Park Service.
223. Food and feeding activities of the salt marsh snail (Melampus bidentatus). Sibyl A. Hausman, Connecticut College for Women. (Introduced by Pauline H. Dederer.)
224. Studies in population physiology. VII. The relation of environmental conditioning to the decline of Tribolium confusum populations. Thomas Park and Nancy Woollcott, Johns Hopkins University.
225. Regional distribution of northeastern Texas acridians. F. B. Isely, Trinity University (Texas).

Miscellaneous

226. Copulation in the acoelous turbellarian Amphiscolops langerhansi. Libbie H. Hyman, American Museum of Natural History.
227. Some cave planarians of the United States. Libbie H. Hyman, American Museum of Natural History.
228. Studies on host reactions to larval parasites. I. Effect on weight. George W. Hunter, 3rd and Wanda S. Hunter, Wesleyan University.
229. Resistant and susceptible groups of white Leghorn chickens to the nematode Ascaridia lineata (Schneider). J. E. Ackert, Ivan Pratt and A. E. Freeman, Jr., Kansas State College.
230. Neurons and connective tissue cells in the enteric walls of Necturus. Elbert C. Cole, Williams College.

ABSTRACTS

An author index of abstracts begins on page 131.

PAPERS READ

Embryology

1. BREEDING HABITS AND DEVELOPMENT OF THE SIAMESE FIGHTING FISH (BETTA SPLENDANS). C. P. Leblond and T. C. Kramer, Yale University. (Introduced by H. B. Goodrich.) (Moving pictures; 15 min.)

This film is divided into two parts. In the first part, views taken from underneath the surface of the water show, 1) the male building a nest of air buddles in which the eggs will be placed and the young maintained after hatching; 2) successive matings followed by extrusion of the eggs which are retrieved to the nest.

The second part shows the development of the embryo from the first segmentation division to the free-swimming stage that is reached about 36 hours after fertilization. In order to take microscopic moving pictures the fertilized egg must be kept in foam, dying if immersed in water. Dorsal and lateral views of the development are shown. The segmentation divisions with expansion and contraction of the blastodisc and yolk, the very rapid envelopment of the yolk by the germ ring and the growth of the embryo are shown at a rate of speed varying from × 720 to × 1000.

Normal speed views of the heart beat, circulation of the blood and changes in the structure of the body after hatching are also shown.

2. THE MICROSCOPICAL ANATOMY OF A HAPLOID, METAMORPHOSED SALAMANDER (TRITON TAENIATUS, LAUR.). Gerhard Fankhauser, Princeton University. (Lantern; 15 min.)

Haploid salamander embryos may be obtained by dividing the fertilized egg into two parts (Spemann, '14). The fragment containing a sperm nucleus, but not the egg nucleus, develops with the haploid, paternal set of chromosomes (merogony). Most of the merogonic embryos die in early stages; relatively few develop into larvae. A single haploid larva has so far been raised to metamorphosis (Baltzer, '22 and Fankhauser). This larva, which was obtained from an egg fragment of Triton taeniatus, was much delayed in its development and dwarfed. It died at the end of metamorphosis, at the age of 100 days.

A preliminary study of the serial sections through this haploid Triton taeniatus shows that the organs are well differentiated but smaller than normal. The individual cells and nuclei appear to be smaller than in a diploid animal of the same stage. The skin has assumed all the characteristics present in a normal, metamorphosed newt. Mitotic figures are found in some of the epidermal cells and make a direct test of the permanent haploidy of the animal possible.

The gonads, although small, present the typical appearance of young ovaries. The germ cells are arranged in a cortical layer around a central cavity; they are, moreover, characterized as oogonia by the typical spireme-like structure of the nuclei.

The advance stage reached by this haploid animal and, particularly, the normal appearance of its organs under the microscope indicate that a sufficient number of experiments may possibly produce viable haploid salamanders.

3. The transplantation of lateral half medullas from Amblystoma tigrinum to A. punctatum. C. Hutchinson, Morris Biological Farm of The Wistar Institute. (Introduced by Helen Dean King.) (Lantern; 10 min.)

The piece of the medullary plate which forms the right lateral half of the medulla was transplanted from embryos of Amblystoma tigrinum to the normal position in similar embryos of A. punctatum. At first the grafts develop at a faster rate and attain a greater size than the corresponding region of the host. Cell counts show that the difference is greatest in late tail bud stages when the graft may have 43% more cells than the corresponding region of the host, though it is only slightly larger than the same region of the donor control. A little later the graft may be 21% larger than the corresponding region of the host and 10% larger than a similar region of the donor control. The grafts then tend to regulate toward the size typical for the host and complete regulation was found in four out of six cases studied at the stage of beginning feeding.

In volume the nervous tissue of the graft is not much greater than similar regions of the host. However, the cartilagenous derivatives of the tigrinum neural crest are much larger than those of the punctatum host. These volume relations have been studied by means of the paper weight method.

4. The action of synthetic male hormones upon the gonads and gonoducts of the chick embryo. B. H. Willier, T. F. Gallagher and F. C. Koch, The Universities of Rochester and Chicago. (Lantern; 15 min.)

The synthetic male hormones—androsterone, dehydroandrosterone and androstenedione—dissolved in propylene glycol, were either injected directly into the albumen of eggs incubated 48 hours, or dropped on the superficial layer of albumen just to one side of the blastoderm of embryos of 60 to 72 hours. Dosages were single and ranged from 0.02 mg. to 1 mg. Development was continued until the sixteenth to eighteenth day. By using embryos with a sex linked plumage character the original sex could be readily identified.

All three of the above-mentioned substances cause a definite modification in the form and structure of the genetic female gonads. The right ovary enlarges into a cylindrical testis-like body consisting of medullary tissue. The left likewise becomes testis-like—narrower and thicker than normal, the cortex more or less thin and the medulla hypertrophied. With high dosages (0.66 mg to 1 mg.) the cortex of the left disappear more or less completely, and definite male sex cords begin to appear in the hilar region of both left and right ovaries. The left oviduct is often rudimentary or absent in both distal and proximal portions, indicating a suppression of development; the right is occasionally increased in length. Wolffian ducts and some mesonephric tubules undergo hypertrophy often to a considerable degree. The genetic males are usually unmodified except for slight thickenings of the germinal epithelium on the left testis, regarded as the beginning of cortex differentiation. Thus a weak feminizing action is indicated. Generally, in producing the variety of effects mentioned above, dehydroandrosterone is the most potent of the three substances tested, androstenedione the least effective.

4A. TOTIPOTENCY OF REGENERATING MESENCHYME IN ADULT AMPHIBIANS. Oscar E. Schotté, Amherst College. (Lantern; 15 min.)

No abstract received.

5. OVUM AGE AND PREGNANCY IN THE GUINEA PIG. DATA FROM 462 ARTIFICIALLY INSEMINATED FEMALES. Richard J. Blandau and William C. Young, Brown University. (Lantern; 15 min.)

In preliminary experiments on the developmental capacity of eggs from 235 animals inseminated approximately 8 to 32 hours after ovulation, the limit of ovum viability was about 26 hours, the limit of ovum age followed by normal development of any individuals was 20 hours, and ovum age when age effects were first expressed was about 8 hours (Science, vol. 84, 1936). Although preparation and study of most the recovered embryos have not yet been possible, it is known that the fertilized old eggs developed into grossly pathological embryos aborted early in pregnancy.

An extension of the study to determine, 1) if some later foetal deaths also are attributable to ovum age, and 2) the precise nature of the defect has involved the insemination of 227 additional animals from a new colony. As a by-product, data to be added to those summarized above have been obtained. These are similar to those from the first experiments except that no normal pregnancies followed fertilization of eggs older than about 14 hours. Seven per cent abnormal pregnancies occurred in the control group, 25% in animals inseminated approximately 8 hours after ovulation, 71% in animals inseminated approximately 14 hours after ovulation, and 100% in the animals inseminated approximately 20 and 26 hours after ovulation. Consequently, the conclusion that ovum age is important in determining the character of development and the course of pregnancy in this species is confirmed. The question of the time of abortion in abnormal pregnancies of this type will be discussed.

6. THE DEVELOPMENT OF THE RAT EGG AFTER ITS IMPLANTATION IN A FOREIGN CAVITY. J. S. Nicholas, Yale University. (Lantern; 15 min.)

It has previously been reported that the sensitized uterus will support the development of the rat egg into a foetus, that the serous surface of the mesentery will support development, and that the timing mechanism of birth is correlated with age of egg rather than with time of initial uterine sensitization. These phenomena have been studied by a combined manipulation of the egg and the uterus by experimental intervention.

The present experiments substitute a piece of intestine for a segment of uterus. Intestine from the region near the ilio-cecal junction was anastomosed with its own vascular pedicle into the uterus. The gut heals readily to the cut ends of the uterus and continuity is established in a comparatively short time. Patency of the lumen occurs at the end of the second week. One week later the animals were mated. Pregnancy ensued on the first mating, the embryos generally implanted in the portions of the uterus above and below the introduced gut. Their presence there showed that materials present in the gut or secreted by it had no decidedly deleterious effect upon the passage of the sperm or eggs.

Embryonic growth and differentiation can occur in the transplanted gut either by implantation into the uterine mucosa regenerated from the cut ends or by implantation into the submucosa of the deepitheliized gut surface. The foetuses developing in the transplanted gut surpass those developing in the uterine mucosa both in size and differentiation.

7. THE PARTHENOGENETIC ACTIVATION OF RABBIT EGGS. Gregory Pincus, Harvard University. (Lantern; 15 min.)

Rabbit ova activated by treatment with hypertonic solution and heat have been examined during the course of their development in vitro and after transplantation in vivo. Similar studies have been made with rabbit eggs activated by rat sperm. The initial cytological changes occurring in such ova may involve, 1) the formation of the polar bodies and inward migration of the spindle with subsequent haploid or 2) the suppression of polar body extrusion and the formation of a diploid egg either by fusion of the elements of the polar telophase or by formation of somatic chromosomes with suppression of meiosis. With the methods of treatment employed only a certain proportion of the ova cleave and a lesser proportion enter the growth stage. The cytology of such ova will be described and their fate upon transplantation into the oviducts.

8. THE FACTORS CONTROLLING BLASTOCYST GROWTH IN THE RABBIT. Gregory Pincus and N. T. Werthessen, Harvard University. (Introduced by W. E. Castle.) (Lantern and motion picture; 15 min.)

In rabbits ovariectomized shortly after fertilization ovum growth ceases in early blastocyst stages upon the entry of the ova into the uterus. Progestin injection into such rabbits promotes the growth of the ova and the growth of the eggs is proportional to the hormone dosage employed. There is a good correlation between extent of endometrial proliferation and blastocyst growth. Ova removed from a rabbit doe and placed in culture will cleave but they collapse shortly after entry into the blastocyst stage under ordinary conditions of culture. When the amount of medium (blood serum) is increased to many thousand times the volume of the egg blastocyst collapse will not occur. Rabbit morulae have been carried through to implantation stages by this technique, the best growth having been attained in an apparatus especially designed to permit the circulation and aeration of the medium. Ova in arrested blastocyst stages taken from ovariectomized females have grown into late blastocyst stages in culture. It is concluded that the pseudopregnant uterus produces special uterine conditions necessary for blastocyst growth and the developmental inhibition after ovariectomy is due to these and not to any change in the ovum itself.

9. THE DEVELOPMENT OF A SALAMANDER (AMBLYSTOMA PUNCTATUM). L. S. Stone and T. C. Kramer, Yale University. (Demonstration paper introduced by Edgar Allen.) (Moving pictures; 15 min.)

A detailed story of the development of Amblystoma punctatum from the one cell to the feeding stage has been assembled in 1000 feet of 35 mm. motion picture film. The rate of speed at which the pictures were taken was varied (× 300 to × 8000) so that a more steady change in development is shown upon the screen. The time lapse in the detailed development is about 4 weeks in a temperature environment of about 70°F.

Details of cell division, the formation and closure of the blastopore (ventral view through Zeiss prisms), the formation and closure of the neural folds and other periods of development cover the Harrison stages. Periods are clearly shown in which shifts in the position of the center of gravity, the movement of cilia and motor development move the egg or embryo.

The film begins with a view of the typical habitat in which the animals live and lay their eggs. It ends with a view comparing the size of a full grown adult with various stages in earlier development. Scenes showing the first feeding reactions and circulation of the blood in a larva are also shown in appropriate sections of the film.

The Baldwin-Patten moving picture apparatus (Patten and Kramer, Anat. Rec., vol. 52, '32) was used.

Embryology papers continued with no. 27.

Physiology

10. NARCOSIS OF FROG MUSCLE BY N-BUTYL ALCOHOL. Robert W. Ramsey, University of Rochester. (Lantern; 15 min.)

The maximum tension exerted by a stimulated frog sartorius muscle is reduced when in the presence of solutions of n-butyl alcohol. It is necessary to determine whether the tension measured at any particular depth of narcosis represents: a) the sum of the tensions produced by each and every fiber—all of which have been partially reduced by action of the narcotic or b) a given fraction of the total number of fibers left contracting, the remainder being inexcitable. The latter was tested by measuring minimum alpha rheobases against time with a range of concentrations of the alcohol. All concentrations below those which gave 100% reduction of tension caused a slow rise in rheobase to a maximum of 4.5 times normal. At greater concentrations the rheobase increased slowly for a short period and then rapidly rose to infinity. Attempts to maintain normal maximal tension during narcosis by raising the stimulating voltage, were only partially successful. Nevertheless it would seem that the per cent loss of tension occasioned by a given concentration of alcohol is due to the complete inexcitability of approximately the same fraction of the total number of fibers in the muscle.

Therefore both the kinetics and the equilibrium values depend upon the strength of current used to excite. Under a fixed set of conditions the limiting factor in the kinetics was the rate of diffusion of the alcohol into the muscle. No simple relation was observed between concentration and final per cent action of the narcotic.

11. THE INJECTION OF ACETYLCHOLINE AND OF OTHER AQUEOUS SOLUTIONS INTO ISOLATED MUSCLE FIBERS OF THE FROG. Elsa M. Keil and F. J. M. Sichel, New Jersey College for Women (Rutgers University) and Howard University School of Medicine. (15 min.)

Recent demonstrations of the role of acetylcholine in neuromuscular transmission in mammals suggested the advisability of reinvestigating frog's muscle by microinjection into isolated fibers (Sichel, '34). The results are interpretable only by comparison with microinjections of other aqueous solutions.

Small amounts (5000 μ^3) of distilled water, Ringer, isotonic sucrose, etc., when injected into or blown upon the surface of the isolated fiber (adductor of leg) produce only purely passive visible effects. This is true only if clean, freshly washed micropipettes be used. If larger quantities be injected, or if the injection be very rapid, or if a previously used pipette be employed, a localized contraction of the interior of the fiber occurs which is immediately reversible on stopping the injection. Coincident with the contraction, the fibrillar structure becomes sharply visible at the pipette tip. The contraction is accompanied by diminished inter-striae distances.

$CaCl_2$ causes a violent shortening of the fibrillar material (Chambers and Hale). This contraction is reversible and occurs even when minimal amounts of M/200 solution are injected.

Application or injection of acetylcholine in concentrations from 1 in 10^3 to 1 in 10^{11} has no visible effect, even after eserine, beyond the effects attributable to distilled water, Ringer's solution, etc.

The preparation can give a twitch response to an electrical stimulus although it is incapable of a conducted response, and is apparently denervated (Brown and Sichel, '30, '36). We conclude that acetylcholine does not act directly upon the contractile mechanism in frog's muscle.

12. POTENTIALS DEVELOPED IN CONTRACTIONS OF THE BODY WALL OF LUMBRICUS TERRESTRIS. Robert Galambos and Paul Frederick Brown, Oberlin College. (Introduced by R. A. Budington.) (Lantern; 15 min.)

Oscillograms and kymograms of potentials correlated with contractions of the earthworm body wall are presented. A method for recording these potentials with the kymograph alone, as well as simultaneously with kymograph and oscillograph is described.

The potentials developed in this contracting smooth muscle appear before the movement begins and continue through the first part of the myogram. No movement occurs without accompanying potential, and an increase in the amplitude of the recorded potential accompanies an increase in the amplitude of the contraction.

13. EXCITATION OF THE GIANT FIBER SYSTEM IN THE SQUID, LOLIGO PEALEI. C. Ladd Prosser, John Z. Young and A. H. Chambers, Marine Biological Laboratory, Woods Hole. (Lantern; 10 min.)

Excitation of the giant fiber in the great stellar nerve of the squid elicits an all-or-none contraction of the mantle. Strength duration curves for this response were obtained by means of condenser discharges. The curves and excitation constants derived are essentially similar whether the giant fiber is isolated or is contained in the nerve trunk. With chlorided silver wire electrodes, 0.6 mm. in diameter, 3 mm. apart, chronaxies range from 0.05 to 0.22 ms. and characteristic times (slope one-half on log-log plot) range from 0.12 to 0.83 ms. Similar values are obtained with pore electrodes; but with silver plates 7 mm. in diameter the time constants are greatly increased. The constants for the giant fibers of other stellar nerves are similar. Log-log plots follow Hill's theoretical equation well.

The fin nerve fibers are 2 to 10 μ in diameter; the fiant fibers 200 to 800 μ. The fin nerve fibers have chronaxies from 0.28 to 0.56 ms. and characteristic times of 1.00 to 2.8 ms.

When the giant fiber is stimulated repetitively the tension developed by the mantle muscle increases slightly (1 to 10%) up to fusion (approximately 35 per second). Above this there is no increase in tension with increasing frequency unless the preparation is irreversibly fatigued. This is in sharp contrast to crab neuromuscular systems.

14. ARE THERE ANTIDROMIC RESPONSES IN THE MELANOPHORE SYSTEM? G. H. Parker, Harvard University. (Lantern; 15 min.)

When melanophore nerves are cut in the tail of a pale Fundulus a dark band forms from the cut distally but none proximally as would be expected if there were antidromic activity. This proximal extension is believed to be checked through the activity of the concentrating nerve fibers acting against the dispersing nerve fibers proximal to the cut. This hypothesis can be tested by inducing the formation of a dark band and, after it has mostly faded, by cutting it again midway its length. It is then found that the newly excited band extends not simply from the new cut to the edge of the tail but also from this cut proximally to the old cut. This proximal extension takes place in consequence of antidromic action on the part of the dispersing nerve fibers, an action which is possible when these fibers are free from the opposition exerted by the concentrating nerve fibers. Melanophore nerves are like motor nerves in that they exhibit antidromic responses (axon reflexes).

15. ELIMINATION OF NEUTRAL RED BY THE FROG'S KIDNEY. Robert Chambers and Rudolph T. Kempton, Washington Square College, New York University and Princeton University. (Lantern; 8 min.)

In the transport of neutral red into the urine from perfusion fluids previous investigators have neglected to consider the effect of pH and have ascribed a secretory activity to the tubular cells in accumulating the dye within the tubules. From vital staining experiments it is known that neutral red diffuses into and out of living cells according to whether the environing medium is more acid or more alkaline, respectively, than the cell interior. By applying this principle to the kidney we have been able to show that artificially alkalinizing or acidifying the urine inhibits or accelerates the elimination of neutral red. This holds true irrespective of whether the neutral red is delivered to the kidney by way of the aortic or the portal circulation.

16. EXCRETORY PRODUCTS OF THE MEDITERRANEAN FLOUR MOTH LARVA (EPHESTIA KÜHNIELLA ZELLER). Nellie M. Payne, University of Minnesota. (Lantern; 15 min.)

Excretory products of the Mediterranean flour moth larva include water, carbon dioxide, urea, uric acid and ammonia. Just before pupation water and uric acid are given off in considerable quantities. The amount of ammonia given off per gram of protein eaten varies with the temperature and with the density of population of the larvae. Crowded larvae tend to produce more ammonia than do larvae reared in separate containers with adequate space. Nitrogen distribution in the excretions is compared with that in the food.

17. NUTRITION STUDIES WITH THE COCKROACH, BLATTELLA GERMANICA. R. M. Melampy and L. A. Maynard, Cornell University. (Introduced by Wm. H. Gatts.) (Lantern; 12 min.)

Diets composed of milk and its products which permit a young mammal to develop were unsatisfactory for the growing roach. This insect responded equally well to a diet of ground wheat and dried skim milk and the same cereal with meat products. The ether extract of wheat furnished a growth stimulating substance which withstood saponification and the unsaponifiable fraction possessed the greater activity. Neither lard nor butter oil could be substituted for the wheat extract. The vitamin B complex of dried yeast required by Blattella germanica was heat and alkali stable. A purified diet of a low fat content was developed which permitted satisfactory development of the growing cockroach. Blattella germanica was able to convert protein into body lipid. The nymphs had the largest amounts of body fat while the females possess two and one-half times as much lipid as the males. In starvation, the amount of fat was not greatly reduced although the iodine number was diminished. The degree of saturation of tissue fat was altered by feeding fats of different iodine numbers.

18. THE CLEAVAGE OF ARBACIA EGGS UNDER HYDROSTATIC COMPRESSION. Douglas Marsland, Washington Square College, New York University. (Introduced by Robert Chambers.) (Lantern; 7 min.)

A special chamber has been constructed, in which cells and tissues may be observed, at a magnification of 300 diameters, while they are exposed to pressures up to 500 atmospheres.

When high (above 300 atmospheres) pressure is applied shortly after the cleavage furrow has begun to constrict the egg, further constriction is stopped and the furrow gradually recedes. If the eggs are decompressed at any time within 15 minutes of the first appearance of the furrow, this constriction reappears and cleavage goes ahead to completion. However, if the eggs are decompressed somewhat later than this, the first cleavage fails to occur, and at the time of second cleavage, the egg divides directly into four blastomeres.

Pressure below 300 atmospheres is chiefly effective in reducing the rate at which the furrow 'cuts' through the equator of the egg. The magnitude of this effect appears to be related to a change in the protoplasmic viscosity which pressure induces in the cortex of the egg.

19. EFFECT OF AGE ON ARBACIA EGG MEMBRANE. A. J. GOLDFORB, College of the City of New York. (Lantern; 10 min.)

At the same osmotic pressure, temperature and pH, freshly shed eggs from different Arbacia cytolyzed at rates that varied by 100%. Under the same conditions eggs from a given female burst progressively much faster with age.

The swelling curve is tripartite, including a primary rapid change, equilibrium and secondary swelling.

The amount of primary or total swelling and membrane stretching did not increase significantly with age. The stretching rates increased markedly with age.

This increased rate was concomitant with increased permeability to water.

The progressively faster bursting with age is attributed chiefly to increased permeability and disproportionately increased stretching rates of the plasma membrane. The same phenomena occurred by increasing the osmotic pressure at a given age. Tensile strength and elasticity seem to play no or a minor role.

Physiology papers continued with no. 32.

Biological effects of radiation

20. The isolation of desired qualities of radiant energy for biological experimentation. Loyd A. Jones and C. Tuttle, Kodak Research Laboratories. (Introduced by the Secretary.) (Lantern; 15 min.)

Many sources commonly used in experimentation on the biological effects of radiant energy emit energy over a very wide range of wave lengths or frequencies. This range may extend in the case of some sources from well into the ultraviolet region at approximately 0.2 μ to the far infrared at 10 or even 30 μ. Of this range only a small region, 0.4 to 0.76 μ, is visible. It is obvious, therefore, that visual inspection of a sample of radiant energy is an extremely poor criterion as to its wave length composition. It is, of course, entirely unwarranted to assume that invisible radiant energy does not have significant biological effects. It is necessary, therefore, in work of this kind to use sensitive receivers other than the human eye to analyze the quality of the radiant energy. The choice of the source and of a method for separating the emitted radiant energy into more or less homogeneous parts will depend upon the requirements of the particular problem.

Methods of determining the quality and amount of radiant energy throughout the region which the selected source emits are discussed in some detail. A special case involving the use of incandescent tungsten as the source of radiant energy and of selective absorbers for the isolation of certain regions is considered. Numerical data relative to energy levels, areas irradiated, total energy consumption, and spectral purity are given. The problem of dealing with energy reradiated from the selective absorbers and other bodies in the vicinity of the organisms being irradiated is also discussed.

21. Ultraviolet radiations as experimental means for the investigation of protoplasmic behavior. E. E. Just, Howard University. (Lantern; 15 min.)

Study of effects of ultraviolet radiation on cells may be made from point of view of physical science: the cell is treated as physico-chemical system; attempt is made to ascertain the likeness and unlikeness of living and non-living matter with respect to response to radiation. This discussion deals with biological effects of radiation—response of that physico-chemical system organized as protoplasm, the unit of biological science. Physics of radiation does not here enter. With radiation only as experimental means am I concerned.

Biologically, ultraviolet radiation alters morphology and physiology of nucleus complex and cytoplasm. Alteration varies with species of egg and with stage in development of a species egg. These effects are summarized. Effect on surface cytoplasm comes first.

Other experimental means—e.g., varying temperature, salinity—produce similar effects. But ultraviolet is most rapid in action, most easily controlled, most effective as measured by per cent of eggs that respond.

Investigators of a protoplasmic system appreciate that its response to changes in environment expresses system's independent irritability. Knowledge of nerve muscle physiology, of experimental parthenogenesis makes clear that experimental means imitating the normal excitant are not special: no single one is the only that elicits the characteristic response by which the system manifests behavior peculiar to it.

Protoplasmic behavior expresses protoplasmic organization. Structure and function of protoplasm, unit reacting system, still remains the basis for biological study. Ultraviolet radiation is a nice supplementary means for biological attack on protoplasmic behavior.

22. Further studies on the effect of radiation on protoplasm. L. V. Heilbrunn, University of Pennsylvania. (Lantern; 15 min.)

These studies have progressed along three lines:

1. Additional evidence has been obtained to show that ultraviolet radiation causes a release of calcium. This has been demonstrated by direct microchemical analysis of the fluid in contact with isolated muscle fibers.

2. In the case of Amoeba proteus, it has been possible to show that ultraviolet radiation causes a release of fat or lipid from combination with protein.

3. Additional evidence has been obtained for the existence of indirect effects of radiation. When the tissues of various marine invertebrates are exposed to roentgen rays, substances are produced which have an effect on the rate of cell division of the egg cells.

23. Ultraviolet irradiation of microorganisms and some interpretations. B. M. Duggar and Alexander Hollaender, University of Wisconsin. (Introduced by W. C. Curtis.) (Lantern; 20 min.)

In considering the effects of radiation on microorganisms, the following phenomena have been studied: 1) Lethal effects of ultraviolet. A comparison of these effects is made with that of killing by heat, affording suggestions as to the mechanisms. 2) The influence of sublethal doses have thus far brought out two effects which may be characterized as an initial growth period followed by an extended lag phase—both being demonstrated in standard nutrient media. Furthermore, the post-radiation effects of certain salt solutions more sharply emphasize the initial growth phase and the apparently extended lag period and also increase the dying rate.

Methods and procedures which have made it possible to recognize these effects are primarily, a) a source of intense monochromatic light, and appropriate equipment, b) exact quantitative means of following radiation intensity, c) a method of irradiating the organisms in liquid suspensions with the suspending solution non-absorbing at the wave lengths employed, and d) an efficient and statistically safe method of closely-spaced transfer intervals, assuring many points on the growth and dying curves. The absorption spectra of cell compounds which could be responsive to the radiation (such as proteins, nucleic acids, lipoids, polysaccharids, pigments) are considered. In the killing of bacteria a nucleic acid effect is suggested; while in the inactivation of the virus of tobacco mosaic, a protein effect. In the interpretation of radiation influences the bearing of different theories is discussed and application is made to certain genetical effects.

24. A RESPIRATORY STIMULATING FACTOR. John C. Fardon, Robert J. Norris and John R. Loofbourow, Institutum Divi Thomae. (Introduced by W. C. Curtis.) (Lantern; 15 min.)

A respiratory factor was produced from yeast cells either by subjecting them to heat (60°C. to 100°C.), ultraviolet radiations or x-rays.

The first investigations into the nature of this substance showed it to be heat and time-stable. Other cells under like conditions were found to secrete a similar substance, for example, irradiated rat liver pulp stimulated the respiration of yeast suspension while the extract of irradiated yeast cells stimulated the rat liver tissue, but not in the same degree. This difference may indicate a degree of specificity in regard to the kind of tissue.

Some of the properties of the new substance resembled closely those of 'bios.' Not only did it increase oxygen consumption, but it also stimulated fermentation and growth. Its threefold action may eventually disclose the fact that we are dealing with three distinct fractions which stimulate, respectively, respiration, fermentation and growth.

Experiments are now under way to determine the significance of these fractions in cancer.

Perhaps these fractions have a relationship to individual cells comparable to the relationship of hormones and vitamins to gross organisms.

25. INTENSITY DISCRIMINATION AND RESPONSE TO FLICKER. W. J. Crozier, Harvard University. (Introduced by the Secretary.) (15 min.)

No abstract received.

26. RESPONSE TO LIGHT IN PERANEMA TRICHOPHORUM, WITH SPECIAL REFERENCE TO DARK-ADAPTATION AND LIGHT-ADAPTATION. S. O. Mast and Landrum B. Shettles, Johns Hopkins University. (Lantern; 15 min.)

If the illumination is suddenly increased, Peranema responds with rapid deflection of the anterior end. The entire organisms is sensitive to light, the flagellum most, the posterior end least.

The reaction time consists of an exposure period and a latent period. With increase in intensity of illumination, the exposure period decreases at first rapidly, then more and more slowly until it becomes nearly constant while the latent period increases to maximum and then decreases. The product of exposure period and intensity increases from 22,970 m.c. second at 538.2 m.c. to 54,315 m.c. second at 2152.8 m.c., then decreases to 13,498 m.c. at 6458.4 m.c.

With increase in temperature the exposure period decreases, but only slightly while the latent period decreases considerably.

Strychnine causes great decrease and cocaine considerable increase in reaction time.

If light-adapted peranemae are subjected to darkness, then exposed to light of a given intensity, the reaction time decreases to minimum (as dark-adaptation increases), then increases to maximum, then remains nearly constant. The time in darkness required to produce maximum reaction time varies directly with the intensity to which they are adapted.

If dark-adapted peranemae are exposed to light, then subjected to darkness for 30 minutes, then exposed to light of a given intensity, the reaction time decreases to minimum (as light-adaptation increases), then increases to maximum, then remains nearly constant. The time required to reach maximum reaction time varies directly with the adapting intensity.

The maximum stimulating efficiency in the visible spectrum is at wave length 505 ± mμ.

Papers no. 27 to 31 are for the special joint program on Genetics and Development and no abstracts are given.

Physiology (continued)

32. EFFECTS OF VARIOUS IONS ON THE RHYTHMIC MOVEMENTS OF EMBRYOS (ACRIDIDAE, Orthoptera). Vincent Thompson, State University of Iowa. (Introduced by J. H. Bodine.) (Lantern; 10 min.)

A study of the behavior of the lateral wall beatings of the grasshopper embryo, Melanoplus differentialis, in different ionic environments has been made. All solutions were prepared isotonic to Belar. The contractions were unaffected by solutions of pH 3.8 to 10.6. Using the chlorides of the cations, and buffering where possible to pH 6.3, it was shown that, 1) the higher the atomic weight, the more toxic the effect of the ion on the movements; 2) antagonism could be demonstrated between potassium ions and magnesium, calcium, barium, strontium and cadmium ions, 3) solutions containing excess amounts of monovalent ions (Na, K, Rb and Cs) accelerated, while divalent ions decreased the rate of movements. Sodium salts of the anions were employed. The anions may be divided into three groups: 1) those which are apparently without effect on the contractions, and may be used in place of the chlorides; nitrate, sulphate, bromide and iodide; 2) anions in whose solutions the movements cease immediately, but in which recovery may occur: bisulphate, bicarbonate, acetate; 3) extremely toxic anions, as cyanide and fluoride. Tartrate, citrate and oxalate inhibit the contractions in the order named.

33. THE EFFECT OF CERTAIN STIMULATING AND INHIBITING SUBSTANCES ON RESPIRATION OF ACTIVE AND BLOCKED EGGS AND ISOLATED EMBRYOS. J. H. Bodine and E. J. Boell, State University of Iowa. (Lantern; 5 min.)

A study has been made of the oxygen consumption rates of morphologically similar but physiologically different eggs and isolated embryos of the grasshopper, Melanoplus differentialis. Results have been expressed in terms of rates of O_2 uptake per milligram dry weight per hour. Basal metabolic rates are thus available for comparative experimental work with this form. Actively developing embryos have rates of O_2 consumption three times that for similar embryos when in the blocked or diapause state. The effects of O_2 tension, carbon monoxide and cyanide on the egg as well as the naked embryo free from the egg have been studied.

The action of methylene blue on the O_2 uptake of the active and blocked isolated embryos has been investigated. Marked differences in response are shown, indicating that the stimulating action of M.B. is perhaps tied up with the physiological condition of the cell at the time of application. The antagonism of M.B., cyanide, and carbon monoxide have also been studied and compared.

34. TEMPERATURE STUDIES ON THE EGG AND YOUNG OF THE SMOOTH GREEN SNAKE. M. X. Zarrow and C. M. Pomerat, Clark University and Harvard University. (Introduced by A. B. Dawson.) (Lantern; 10 min.)

The oxygen consumption and carbon dioxide production of four eggs and corresponding hatched young of the smooth green snake, Liopeltis vernalis (Harlan) were measured at temperatures ranging from 12°C. to 30°C. by means of a modified Warburg respirometer. A temperature characteristic (μ) of 24,400 ± was obtained for values between 12°C. and 24°C. and another of 16,100 ± for respiration at temperatures from 25° to 30°C. Results for O_2 and CO_2 were essentially similar. The respiration of the hatched snakes showed no shift in temperature characteristics. The absolute rate of gas exchange for the hatched young was somewhat less than that found for the egg even though the weight and volume of the latter was more than 50% greater. No attempt was made to determine the amount of inert material in the eggs or the weight of the embryonic membranes. The mean rate at 23°C. for O_2 consumption was 97.71 cu.mm. per ½ hour for the eggs and 86.12 for the snakes. The average respiratory quotient for the eggs was 0.8227 and was not found to be affected with time nor with changes in temperature. The average quotient rose to 0.9034 for the newly hatched snakes. These findings lend support to the view that the act of hatching per se does not markedly affect the fundamental processes of metabolism which are in progress.

35. A STUDY OF THE VARIATION IN THE METABOLIC RATE OF MAN AND RATS. N. T. Werthessen, Harvard University. (Introduced by T. J. B. Stier.) (Lantern; 8 min.)

To study the metabolic rate of the rat during a 36-hour fast an apparatus was built which gave a continuous measurement of oxygen consumption and an intermittent determination of CO_2 production.

A cycle is found which forms, when rate of oxygen consumption and CO_2 production are plotted against the extent of the fast, a semicircular curve, concave upward. The minimum occurs at about the sixteenth hour—the second maximum is reached by the twenty-fourth. This maximum is followed by an unpredictable variation.

Experiments on four men, using standard apparatus, showed similar results. R.Q. values in all above experiments were normal.

Rats were trained for 2 weeks or longer to eat during a short period once every 24 hours. Only weight gaining rats were used in the experiments. Data obtained were checked by standard apparatus.

These rats show a sharper cycle than normals. The semicircle is changed into a flat bottomed V. Movement per unit period and R.Q. values follow the cycle. R.Q. values begin at 0.8 and drop to 0.3 or lower and rise again to 0.8 or 1.2. These values can be shown to be real and not apparent.

Since the values for the respiratory quotient change in a definite cycle, it follows that the metabolism of the trained rat is based on a chain reaction system and that at the lowest rates obtained a conversion of fat into carbohydrate occurs which is presumably the first reaction of the chain.

36. Red cell fragility in guinea pigs after splenectomy. Albert S. Gordon, William Kleinberg and Eric Ponder, Washington Square College, New York University and The Biological Laboratory, Cold Spring Harbor. (15 min.)

The resistance to hypotonic serum of the cells of guinea pigs defibrinated blood increased after splenectomy, the maximum increase being obtained about 6 weeks after the operation, and amounting to about 0.1 tonicity unit. This marked increase in resistance might be due to a change in, a) the water content of the cells, b) the leakage of salts which occurs in hypotonic solutions, or c) the 'critical volume' at which the cells hemolyse. Determinations of the water content of the cells show that this is unaltered after splenectomy, and diffractometer measurements of the critical volume for commencing hemolysis show that after splenectomy the cells begin to hemolyse when their volume has increased to 145 to 150% of the initial volume, as compared with an increase of 130 to 135% in control animals. These measurements were carried out on cells rendered spherical by means of lecithin. The extent of leakage as measured by the R-value is not significantly altered. The cause of the increased resistance is accordingly an increased extensibility of the red cell membrane.

A similar increase in resistance is observed to hypotonic NaCl. This might be brought about by a reduction in the osmotic pressure of the animal's plasma, as well as by the three factors mentioned above. No change in the depression of freezing point of the plasma can be detected; in this case, too, the increased resistance is to be attributed to a greater extensibility of the cell membrane.

37. Physiological adaptations of animals to high altitudes. F. G. Hall, Duke University. (Lantern; 15 min.)

A comparative study of the physiological effects of altitude on several birds and mammals was made. It was found that those animals that naturally live at high altitudes have blood with an affinity for oxygen greater than that of animals naturally living at lower altitudes. Morphological characteristics of the blood seem to be correlated with ability to make adjustments to changes in altitude. Animals such as the llama and vicuna are able to live actively in an environment quite deficient in oxygen. The ability of hemoglobin to carry oxygen seems to account in part for adaptations of certain birds and mammals to high altitudes.

38. Total dissociation of horse hemoglobin. Jacinto Steinhardt, University of Upsala and Cambridge University. (Introduced by Selig Hecht.) (15 min.)

Native horse hemoglobin, in dilute unbuffered salt solutions at the isoelectric point, is totally dissociated into molecules of half the normal molecular weight, when high concentrations of urea or of other amides are present in the solutions. This conclusion depends on measurements of the sedimentation constant in the Svedberg ultracentrifuge, the diffusion constant by the method of Lamm and Polson, and the partial specific volume. The same amide concentrations are without apparent effect on the molecular weight of another protein, pepsin; the possibility that changes in media affect the theoretical relations used in the calculations is thus eliminated.

Spectroscopic and gas-affinity measurements show that the change in molecular weight is unaccompanied by any evidence of denaturation, although some derivatives of the half-weight are relatively less stabile. As methemoglobin it soon changes to an anomalous parahematin. This can be transformed, by reduction with hydrosulfite, to reduced hemoglobin.

The great effect of amides on protein solubility are considerable at concentrations far below those at which appreciable dissociation occurs. Neither phenomenon appears to be directly related to the dielectric constants of the media which were sometimes greater, sometimes smaller, than those of water; glycine solutions with a greater dielectric constant than the others, were practically without effect on dissociation, and had a smaller influence on solubility.

39. SIMILARITY OF THE EFFECTS OF HEAVY WATER AND LOW TEMPERATURE ON THE ELECTRIC POTENTIAL OF FROG SKIN. T. Cunliffe Barnes, Yale University. (Lantern; 10 min.)

White belly skin from a male Rana pipiens was tied securely over the end of a short glass tube 1.5 cm. internal diameter. (There is some evidence that light effects the potential of pigmented areas of skin.) The tube was placed in two thermostats in succession at 26° and at 10°C. Air adjusted to the required temperature was circulated through the jar containing the skin. The electrodes consisted of cotton soaked in Ringer's solution (pH 8.2) fastened to chlorinated Ag wires leading to the usual potentiometer circuit. The Ringer's solution was made up in either ordinary water or in 95% heavy water. It was found that the fall in potential produced by heavy water was strikingly similar to the fall in potential produced by a drop in temperature. Thus, in preparation giving a peak potential of 38.2 mv. in ordinary Ringer's solution showed a drop to 20.8 mv. in heavy Ringer's solution (both at 26°C.). A preparation giving 34.7 mv. at 26° showed a potential of 18.1 mv. at 10°C. (both in ordinary Ringer's solution). The histological picture of skin treated with heavy water will be briefly described. It is concluded that the electrical properties of frog skin are a result of metabolic processes.

40. ON THE MECHANISM OF THE ULTRAVIOLET ABSORPTION IN LIVING CELLS. B. J. Luyet and P. M. Gehenio, St. Louis University. (Lantern; 10 min.)

A previous investigation has shown that the epidermal cells of some plants absorb the ultraviolet light when they are alive and are transparent when killed. There is some experimental evidence that this property is due to the presence of pigments which are released from their chemical bonds during death. In an attempt to substitute vital stains for pigments in non-pigmented cells (transparent to the ultraviolet) the authors succeeded in inducing the ultraviolet absorption. When killed these cells lose this induced absorption although they take a darker color. The problem of the existence of a chemical bond responsible for the ultraviolet absorption in living cells is discussed.

41. ACTION OF GLYCOSIDES ON HEXOSE OXIDATION BY LUMINOUS BACTERIA. Frank H. Johnson, Princeton University. (Introduced by E. Newton Harvey.) (Lantern; 10 min.)

With the Warburg respirometer, certain glycosides were found to stimulate, others to inhibit, the aerobic oxidation of glucose, fructose, mannose or galactose by the washed cells of luminous bacteria, without affecting their endogenous respiration. The oxidation of different hexoses could be stimulated or retarded by the same glycoside. Oligosaccharides had little action, but methyl hexosides caused strong effects.

42. THE SULPHYDRYL DISTRIBUTION IN AMOEBA PROTEUS AS SHOWN BY THE NITROPRUSSIDE REACTION. H. W. Chalkley, National Institute of Health. (Lantern; 15 min.)

Amoeba proteus gives a definite nitroprusside reaction, indicating the presence of SH groups. In adult interkinetic individuals the nucleus gives a strong N.P. reaction, the cytoplasm a weak one. In dividing individuals the nucleus gives a N.P. reaction in the prophase. With the disappearance of the nuclear membrane at metaphase the reaction in the nucleus vanishes. The nucleus in newly divided cells gives a N.P. reaction equal to that in the cytoplasm but cells from 1 to 2 hours after fission show a nuclear reaction stronger than in the cytoplasm.

Only a trace of the material giving the N.P. reaction is soluble in trichloracetic acid, indicating that all but a trace of the sulphydryl component is fixed in the proteins of the cell. Moribund intact cells give a weak reaction. Cells treated with iodine or H_2O_2 until moribund give a weak or negative reaction indicating that the SH groups may be physiologically active and not of merely structural significance. In such cells the nucleus always gives a stronger reaction than the cytoplasm.

43. PHARMACOLOGICAL STUDIES ON EXTRACTS OF PERIODICAL CICADAS AND OF GRASSHOPPERS. David I. Macht, Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore. (Introduced by George R. LaRue.) (Lantern; 12 min.)

Seventeen-year locusts (Magicicada septendecim) were collected in Baltimore in the spring of 1936, and the sexes were separated. Each insect was then divided in two; that is, the portion containing the abdomen was taken from that comprised of the head and thorax. A pharmacological study of the different fractions, extracted with hot alcohol and chloroform, respectively, was made on both animal and plant test objects. Either the alcoholic extract as such or the residue obtained by evaporating the alcohol and preparing a suspension in physiological saline was employed. A quantity of grasshoppers (Melanoplus differentialis) was similarly extracted with cold and hot alcohol, and pharmacological studies were made with extracts of the different fractions. It was discovered that the insects contained a toxic substance which produced characteristic depression of the circulation, respiration and central nervous system as well as other interesting pharmacological effects. The abdominal fractions were richer in such a toxic substance. Furthermore, there was a quantitative difference in toxicity between male and female fractions. An alcoholic extract of grasshoppers was studied also for its vitamin A content.

Physiology papers continued with no. 54.

Endocrinology

44. EFFECT OF CASTRATION AND OESTRIN TREATMENT ON RELATIVE AMOUNTS OF F.S.H. AND L.H. IN THE HYPOPHYSIS. Samuel L. Leonard, Union College. (Lantern; 15 min.)

By means of the augmentation reaction, determinations were made of the changes in the relative amounts of follicle stimulating (F.S.H.) and luteinizing (L.H.) hormones of the hypophysis which occurred after castration and oestrin treatment in the adult female rat. The test used was based on the fact that the size of the immature rat ovary is determined by the relative amounts of F.S.H. and L.H. injected. By injections of an excess amount of the F.S.H. (from menopause urine) into immature female rats, and then determining the per cent of augmentation when equal amounts of hypophyseal tissue from experimental and control rats were added, differences in augmentation indicated changes in the L.H. in the hypophyseal tissue. Similarly, by substituting pregnancy urine hormone (L.H.) for F.S.H. in the above experiment, changes in the F.S.H. in the hypophysis of the rat were measured. It was found that castration markedly increased the F.S.H. content of the hypophysis but only slightly increased the L.H. Oestrin treatment in the castrated rats decreased both gonad stimulating hormones.

With a slightly different method, it was found that oestrin lowered the L.H. content in the normal immature female rat hypophysis, but did not affect the F.S.H. content. By these methods it is proposed to study the changes in the F.S.H. and L.H. in the hypophysis during the normal oestrous cycle.

45. EXPERIMENTAL STUDIES OF THE ANTERIOR PITUITARY. IV. THE REPLACEMENT CAPACITY AND NON-CYCLIC BEHAVIOR OF HOMOPLASTIC ANTERIOR PITUITARY GRAFTS IN THE GUINEA PIG. M. Schweizer, H. A. Charipper and H. O. Haterius, Washington Square College, New York University and Ohio State University. (Lantern; 15 min.)

Intraocular implants attached to the pupillary margin of the iris and extraocular implants in the lateral subjunctival tissues become predominantly basophilic and retain their follicle stimulating capacity in completely hypophysectomized guinea pigs. The ovaries become progressively more follicular until after approximately 2 months a condition of constant oestrus supervenes. This condition is characterized by a persistent vaginal opening, a markedly hypertrophied uterus and by well proliferated mammary glands. The ovarian effects of such implants is, however, a purely follicle stimulating one. The ovaries contain no well-defined luteal bodies and the endometrial pattern is typically that brought about by oestrin influence. The complete absence of luteinizing hormone is further attested by the absence of fully mature follicles and of ovulation.

This loss of the cyclic character of the guinea pig pituitary when transplanted is, in all probability, due primarily to the severance of its normal neural connection.

46. EXPERIMENTAL OVULATION AND PSEUDOPREGNANCY IN THE HYPOPHYSECTOMIZED RABBIT AFTER PARTIAL GONADAL REGRESSION. Mark A. Foster, Harvard University. (Lantern; 15 min.)

Within a few days after removal of the hypophysis large doses of pituitary extracts administered intravenously failed to elicit ovulation, but after follicular growth was induced as a result of follicle-stimulating pituitary extract (F.S.H.) treatment over a period of 5 days, ovulation could be produced by an intravenous injection of F.S.H. plus a trace of luteinizing hormone (L.H.). The number of ovulation points varied with the interval between hypophysectomy and the first injection of F.S.H. When treatment was begun before 14 days one to eighteen ovulation points in each animal resulted, while after this time the ovulation reaction rapidly failed. No attempts at ovulation were successful when treatment was begun later than 23 days after hypophysectomy.

Ovulation was followed by atrophy of the granulosa cells with little or no development of lutein tissue. When F.S.H. was administered after ovulation, the ruptured follicles failed to develop lutein tissue and the uteri remained in an oestrous condition. However, after administration of L.H. or a combination of the two hormones, functional corpora lutea developed as shown by a maximal pseudopregnancy reaction in the uterus.

These studies suggest a synergistic action between F.S.H. and small quantities of L.H. in the induction of ovulation and corpus luteum formation.

47. FOLLICULAR GROWTH AND OVULATION UNDER EXPERIMENTAL CONDITIONS IN THE GUINEA PIG. Edward W. Dempsey and William C. Young, Brown University. (Introduced by A. D. Mead.) (Lantern; 15 min.)

Determinations of follicular volumes have shown that the follicular growth rate is not significantly altered after removal of the corpus luteum by unilateral ovariectomy when unilateral ovulation has occurred, by injection of progesterone, by the intervention of pregnancy or by injections of oestrin.

Removal of the corpus luteum hastens ovulation significantly, shortening the cycle to an average of 11.3 days. The growth rate of the follicles is the same but ovulation occurs when the follicles are smaller than normal. Injections of 0.02 I.U. progesterone daily into five animals did not alter the follicular growth rate although the preovulatory swelling and ovulation did not occur. Such follicles are capable of ovulation because discontinuance of the progesterone treatment was followed by ovulation in 2 or 3 days (five animals) and injections of 10 to 25 R.U. antophysin produced ovulation in six animals within 24 hours. Similarly, during the first 12 days of pregnancy, the follicular growth rate is normal and thereafter follicles of this size are always present.

Injections of 250 I.U. oestrin daily into five animals did not prevent follicular growth, and similar injections did not alter the growth rate in five animals whose developing corpora lutea had been removed by unilateral ovariectomy within 24 hours after ovulation. In the latter animals atresia occurred about the tenth day, the time at which ovulation occurred in the uninjected controls.

Similar data on follicular size after testosterone treatment will be presented.

48. ACCENTUATION OF THE GROWTH EFFECT OF THEELIN ON GENITAL TISSUES BY ARREST OF MITOSIS WITH COLCHECIN. Edgar Allen, G. M. Smith and W. U. Gardner, Yale University. (Lantern; 15 min.)

The outstanding effect of theelin is production of rapid growth in the epithelial tissues of vagina, uterus and mammary glands. Estimations of rapidity of growth by counts of mitotic figures are unsatisfactory because brief periods of cell division are interspersed with long periods of rest, as noted in motion pictures of segmenting ova of rabbit (Lewis) and amblystoma (Stone). Fortunate sections may catch these pulses of mitosis, but more frequently they are missed. By the use of colchecin as developed by Dustin and by Ludford a method is at hand of arresting dividing cells in metaphase, thus producing an accumulation of mitoses for several hours.

Using a dosage calculated to accumulate cell divisions for 8 to 10 hours, a restudy has been made of experimental oestrus in the mouse. Mice ovariectomized for 2 months were injected with 10 rat units of theelin in oil and then sacrificed at intervals up to 48 hours. Nine and one-half hours before killing, colchecin was injected. Sections of the genital organs show 1) the total tissue grown in response to theelin and 2) the number of cells dividing in the terminal period.

The number of mitotic figures accumulated was beyond expectations. This method shows clearly the beginning, sequence and distribution of growth activity in the tissues and stresses the importance of the epithelial reaction.

49. INTERSTITIAL CELL HYPERTROPHY IN THE TESTES OF MICE RECEIVING ESTROGENIC HORMONES. W. U. Gardner, Yale University. (Introduced by Ross G. Harrison.) (Lantern; 15 min.)

Impairment of testicular function following the injection of estrogenic hormones was explained by a reciprocal gonad-pituitary relationship by Moore and Price ('32). The gonad-pituitary relationship has been further extended to explain the mechanism of the sex cycle in rats. Under certain conditions the interstitial cells of the testes increase in number and size following the injections of estrogenic hormones in at least one strain of mice.

Fifteen male mice from the A strain were given weekly injections of 500 i.u. hydroxyestrin benzoate for periods of 76 to 250 days starting at from 7 to 32 days of age. Variable amounts of interstitial cell hypertrophy occurred in all except one animal. The testes were of normal or approximately normal size and yellowish brown in color. Though the tubules were variably damaged a marked hypertrophy and hyperplasia of the glandular vacuolated interstitial cells occurred, widely separating the tubules. In six male mice of the same strain receiving weekly injections of 0.1 mg. of equilin benzoate for 16 to 34 weeks large areas of tubules were completely replaced by these cells.

The testes of male mice of three other strains similarly treated have all shown marked decrease in size, comparable damage of the tubules but absence of interstitial cell hypertrophy.

The seminal vesicles and prostates of all of the mice were similar in showing the effects of estrogenic hormones—namely smooth muscle hypertrophy and epithelial metaplasia.

50. PITUITARY-LIKE EFFECTS OF YEAST EXTRACTS. Frederick L. Hisaw, R. O. Greep and H. L. Fevold, Harvard University. (Lantern; 15 min.)

Acetone dried brewer's yeast extracted with 50% pyridine and 33% acetone yields a water soluble substance which has some hypophyseal-like properties. The testicles of hypophysectomized immature male rats treated with these extracts, soon after the operation, remain within the scrotum and continue to increase in size. The accessory male structures, however, regress practically to the hypophysectomized condition. Histologically the testicles resemble those of normal rats except the interstitial cells show some degeneration.

Treatment of hypophysectomized adult male rats yielded the most striking results. The testes remained scrotal and were sustained in an apparently normal condition throughout the 16 days of treatment. The accessory structures became somewhat smaller than those of normal rats, but were secreting, and were many times larger than hypophysectomized controls. Spermatozoa were abundant in the tubules and epididymes of the treated rats and spermatogenic activity was indicated by the presence of spermatocytes and spermatids.

The testicles of immature pigeons also undergo a moderate hypertrophy with yeast treatment which appears to be due primarily to tubular development.

Boiling the yeast extract for several hours destroys its testis stimulating ability.

These extracts by themselves apparently do not affect the immature rat ovary but when combined with FSH produce marked augmentation. Pregnancy urine, however, is not augmented by yeast extract. Feeding yeast likewise does not augment FSH action.

Ovulation and the production of large hemorrhagic follicles follows the injections of yeast preparations into oestrous rabbits.

Extracts prepared from fresh yeast give similar results but are somewhat less effective.

51. THE INDUCTION OF BROODING BEHAVIOR IN THE JEWEL FISH. G. K. Noble, K. F. Kumpf and V. N. Billings, American Museum of Natural History. (Lantern; 15 min.)

Brooding in the cichlid Hemichromis bimaculatus consists of, a) guarding of eggs and young; b) eating of fish of same size as young but of different species; and c) abrupt 'calling' movements of the fins. Fish of both sexes which have passed through a breeding cycle may be induced to brood by injections of prolactin or pituitary extracts (sheep or fish). Fish at the beginning of the cycle require less prolactin than those ready to spawn, and castrates require less than controls. Males have a lower threshold for prolactin than females. Fish isolated from their eggs until they become non-broody require more prolactin than fish similarly isolated from young.

Brooding may be induced also by corpus luteum extracts and to a less extent by thyroxin and by phenol in 0.5% solution. Controls injected with 0.65 NaCl solutions (thirty-five cases) failed to respond.

Sexually mature fish which have never spawned cannot be induced to brood by any of these agents. Neither castrated nor normal fish of this category would respond to prolactin (twenty cases) nor to corpus luteum extracts (twenty cases). Fish which had laid eggs but never brooded could be induced to brood with prolactin in five out of eighteen cases. It thus appears that one spawning must have taken place before brooding can be induced in these fishes. A previous brooding augments the effect of prolactin but is not necessary.

52. Reciprocal weight changes in liver and testis of pigeons during reproduction. J. P. Schooley and Oscar Riddle, Carnegie Institution. (Lantern; 8 min.)

From ninety-five healthy male pigeons not incubating eggs and not feeding young the normal weight of the liver and testes was obtained. In twenty-seven similar males it was found that the liver weight was increased (12%) and testis weight was decreased (11%) during 2 to 18 days of incubation. In twenty-one males these changes were maintained or accentuated during the succeeding phase of rearing (crop feeding) of young. A histological study of these repressed testes shows sperm formation is diminished.

The significance of these results rests on the circumstance that the development and functioning of the crop sacs of these pigeons showed a release of the pituitary hormone, prolactin, during most or all of the period covered by these tests; and by the fact that injections of that hormone into immature or non-reproducing pigeons has been found in other studies to increase liver weight and greatly diminish testis size. It is therefore probable that prolactin is responsible for both testis repression and liver enlargement during the two above-mentioned phases of the normal reproductive cycle.

53. Sex hormones and serum calcium. Oscar Riddle and Louis B. Dotti, Carnegie Institution. (Lantern; 7 min.)

Earlier work has shown that in pigeons, fowl, toads and fish the serum calcium of females is increased at the period of ripening or ovulation of eggs. With ovarian extracts Shapiro and Zwarenstein increased the serum calcium of Xenopus, but thought it unlikely that the female sex hormone (theelin) was the active agent. As a part of a larger study it is here shown that theelin and some related compounds—in relatively heavy and prolonged dosage—readily increase the serum calcium in male and female birds and mammals. We have used normal doves and pigeons; hypophysectomized, thyroidectomized or castrate pigeons; hypophysectomized-castrate pigeons; normal, hypophysectomized, thyroidectomized and castrate rats; normal dogs. Most pronounced increases (to 28 mg. per 100 cc.) are obtained with pigeons (injected for 7 days).

Using pure preparations in oil it is found that theelin and dihydrotheelin-benzoate are relatively (to estrogenic assay) very potent; dihydrotheelin and theelol have little potency. Progresterone has a low activity, demonstrable apparently only in birds. Androstendiol, and the male hormones, testosterone and testosterone-oxime, even when given in huge doses (2.0 mg. daily for 7 days), have no effect on the serum calcium of either pigeons or rats.

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Animal Behavior and Physiology

54. The place of origin and manipulation of cnidoblasts by Chlorohydra viridissima.

Wm. A. Kepner, R. A. Goodman and A. Ingles, University of Virginia. (Opaque projection; 15 min.)

Immature cnidoblasts are delivered into the endodermal cells by the ectoderm. The endoderm discharges the immature cnidoblasts into the coelenteric cavity. Herein they drift with the currents of the gastric fluid; but are early ingested by the endodermal cells. The endoderm of the body-proper, except that of basal disc, appropriates, for the most part, only penetrants and streptolines. The endoderm of the peristome appropriates many penetrants and streptolines. The endoderm of the tentacles ingests all four types of cnidoblasts. These young ingested cnidoblasts are delivered to the ectoderm, wherein they become orientated and develop a cnidocil in each case. Young cnidoblasts may be transported within 'endogenous cytoplasmic masses' that had been abstricted from the endodermal cells. This indicates that they are being manipulated as objects of food are sometimes dealt with by endodermal cells of hydra. While a given endoderm may be thus ingesting and transporting young cnidoblasts it may also be ingesting senile or used cnidoblasts. These latter are digested. Hydra, therefore, deals with young cnidoblasts in a manner that differs from that in which the senile ones are handled.

The distribution of cnidoblasts depends primarily upon the activity of the other cells of the polyp's body. The cnidoblast's activity is confined to the elaboration of the nematocyst and perhaps also to the formation of the cnidocil.

55. Reactions of Limulus to illuminated fields of different area and flicker frequency.

Ernst Wolf, Harvard University. (Introduced by Leigh Hoadley.) (Lantern; 10 min.)

Young Limulus freshly collected show a strongly positive reaction toward light. Single individuals exposed to the influence of two illuminated fields equal in area but differing in flicker frequency (below fusion) will show a greater tendency to move to the faster flickering field than to the slower. In a sufficiently great number of tests the number of reactions to the fields is proportional to their flicker frequencies.

The same was found for the honey bee. With the bee we were able to balance the effect of two flickering fields by reducing the area of the faster flickering field. For Limulus we found that the areas must be inversely proportional to the flicker frequencies for equal stimulating effects. Since with the greater visual field a greater number of elements is concerned in vision compared with a smaller field, and equal effects can be obtained by adjusting flicker frequencies, it seems probable that for the compound eye of arthropods it is irrelevant whether a greater number of photic receptors is stimulated at a low frequency or a smaller number more often per unit time, or whether a larger number of low frequency impulses travel over many pathways to the CNS or more frequent impulses over fewer fibers.

56. The relative sensitivity to light of ommatidia in various parts of the compound eye of the drone fly, Eristalis tenax. William L. Dolley, Jr. and Mary Alice Hartwell, University of Buffalo. (Lantern; 15 min.)

According to previous investigators the sensitivity to light of the ommatidia in the compound eye of the drone fly increases from the anterior to the posterior end. No actual measurements of the sensitivity of the more posteriorly located ommatidia have been made, however, in any insect. Such measurements for Eristalis are reported in this paper.

The flies were oriented on a turntable in a horizontal beam of light facing the source. The angle through which it was necessary to rotate the table in order to maintain this orientation, when at the same time a given ommatidium was stimulated by light of a given intensity from another source, was used as a measure of the sensitivity of this ommatidium. In other experiments other ommatidia were similarly stimulated by light of various intensities. The relation between the intensity of the light stimulating two ommatidia when equal degrees of rotation was induced was used as a measure of the relative sensitivity of the two ommatidia. In this way the relative sensitivity of various ommatidia was ascertained.

The results obtained show that, as one proceeds from the anterior to the posterior end along a horizontal line through the middle of the compound eye, the sensitivity of the ommatidia rapidly increases to a maximum at about the center of the eye and then decreases at first rapidly and later more slowly to a minimum at the posterior edge.

57. Responses to light in the earthworm, Pheretima sp. Charles D. Howell, Johns Hopkins University. (Introduced by W. G. Lynn.) (Lantern; 15 min.)

Four types of responses to light are given by Pheretima depending upon the nature of the stimulation and the condition of the specimen: increase in activity, contraction of the body and reversal in the direction of locomotion, orientation and local contraction.

Pheretima is negative in lateral illumination in light of all intensities to which it responds, but is more strongly negative in high than in low intensities. Ninety-nine and five-tenths per cent of the responses are negative in 21.53 m.c., but only 62.5% are negative in 0.00215 m.c. It is not positive in low intensities as are Eisenia and Lumbricus.

Excessive stimulation, low temperatures, long exposure to light, or operations on the nervous system reduce the proportion of negative responses, but do not make the animal positive even in illumination as weak as 0.108 m.c. However, removing the brain, or severing the transverse commissure in the brain, or both circumpharyngeal connectives makes them positive in this intensity.

The negative response is dependent upon the crossing-over of sensory-motor nerve fibers in the brain in accord with Prosser's views.

The anterior end is most sensitive to light, the tail less sensitive, and the middle of the body and the large proboscis least sensitive.

Pheretima has photoreceptor cells similar to those found by Hess in Lumbricus, but they are found only in the epidermis. There are none in enlargements of the nerves.

58. NOCTURNAL ACTIVITY RHYTHM IN BATS UNDER CONSTANT EXTERNAL CONDITIONS.

J. H. Welsh and D. R. Griffin, Harvard University. (Lantern; 10 min.)

Records of the general activity of a number of bats (Myotis lucifugus lucifugus), taken from a summer colony, were obtained in the laboratory under normal light conditions. The records showed that the bats had an active period beginning at about 8.00 P.M. and lasting for 2 to 3 hours. When placed in constant darkness this periodic activity continued during the same hours for 4 days.

To determine whether this periodicity would persist for a longer time, activity records were taken of two Pipistrellus subflavus during the last 2 weeks in October. These bats came from caves in western Massachusetts where they had already begun their hibernation. The experiments were conducted in a sound proof room where conditions of light, temperature and humidity were relatively constant. One of these bats showed a daily period of activity at about 7.00 to 10.00 P.M. during the entire 2 weeks. The other showed periods of activity recurring in 24-hour cycles but the time of occurrence was related to the disturbance attending the necessary changing of records.

This type of activity rhythm which persists under constant external conditions has been found in Peromyscus and Microtus and the laboratory white rat among rodents, and in several groups of invertebrates. These experiments indicate that is also characteristic of two genera of bats.

59. EFFECTS OF VARIOUS PERIODS OF STARVATION ON GROWTH, REPRODUCTION, HEART RATE AND LONGEVITY IN CLADOCERA.

Lester Ingle, Brown University and The University of Chicago. (Introduced by A. M. Banta.) (Lantern; 15 min.)

Daphnia longispina, semi-starved for seventeen instars (approximately 29 days) measured at that time 2.26 mm. in length as compared with 2.82 mm. for their well-fed sisters. When these semi-starved mothers were placed in normal manure infusion medium at the eighteenth instar they grew rapidly, their maximum body length being 2.82 mm. as compared with 2.85 mm., the maximum length for well-fed animals. The reproductive rate, which was very low during the period of starvation, increased rapidly when an abundance of food was made available. This treatment resulted in a significant increase in life span; the individuals semi-starved for seventeen instars lived on the average 48.9 days, while their well-fed sisters averaged 30.3 days of life. Starvation throughout life resulted in a life span of about 38 days.

Counts of heart beat frequency showed that well-fed individuals exhibited a frequency after birth of about 4.6 beats per second and reached a peak of 5.5 at the seventh instar. During semi-starvation, mothers exhibited a very low heart rate (approximately 4.3 beats per second) but when introduced into normal medium at the ninth instar, the frequency quickly increased to reach a peak of 5.1 at the fourteenth instar.

A low level of activity in respect to growth, reproduction and heart frequency, as induced by a limited quantity of food, is more conducive for long life than a high rate throughout life or a continued low rate throughout life.

60. INTERPRETATIONS OF DATA ON GROWTH AND REPRODUCTION (DAPHNIA LONGISPINA). Thelma R. Wood and A. M. Banta, Brown University and Carnegie Institution of Washington. (Lantern; 15 min.)

By utilizing the coefficient of correlation we have worked out an unbiased mathematical evaluation of the interdependence of many pairs of variables involved in growth and reproductive activity of 576 individuals from six experimental series of parthenogenetically produced Daphnia longispina, all of the same clone.

The correlations obtained point to the following conclusions. 1) Animals larger at birth develop no earlier than their smaller sisters but 2) tend to become the larger adults and, 3) with unlimited food, produce more young in their first clutches than their smaller sisters. 4) With unlimited food, the number of young produced is more highly correlated with birth length than with early development. 5) With limited food an animal smaller at birth tends to produce as many young in its first clutch as does its larger sister and 6) early growth is in general independent of birth length; but 7) with unlimited food the correlations suggest that early growth compensates for birth length. 8) Animals which grow more during juvenile instars tend to grow less in the following two instars.

9) Number of young in the first clutch seems to be more closely associated with growth of mothers during early instars than during later instars. 10) In any given instar growth increment is correlated positively with number of eggs simultaneously produced, indicating that egg production does not occur at the expense of growth.

11) Within limits, the more an animal grows, the more frequently it moults—i.e., the shorter are its instars in hours.

61. WATER ABSORBING PROPERTIES OF OLIGOCHAETE TISSUES. Mary Elizabeth Kopenhaver, Lake Forest College. (Introduced by J. Wm. Buchanan.) (10 min.)

An orderly differential in water content is shown in the same body wall tissue located at several regions along the antero-posterior axis of the annelids, Allolobophora caliginosus and Lumbricus terrestris. Portions of fifteen segments were cut from three regions: from the extreme anterior end exclusive of the prostomium, from the region immediately posterior to the clitellum, and from the extreme posterior end. The internal organs were removed, the body wall tissue cleaned, pressed between filter paper, weighed, and dried over sulphuric acid until a constant weight was attained. The average water content for fifty animals of each species was:

	Anterior	*Middle*	*Posterior*
Allolobophora caliginosus	78.1%	79.1%	80.1%
Lumbricus terrestris	79.8%	81.7%	82.24%

The tissues along the axis differ with respect to their ability to appropriate water. When placed in distilled water for 4 to 6 hours, anterior tissues gained more weight than did tissues from the middle and posterior regions. The order of water uptake in hypotonic environment was inverse to the order of water content.

In M/1000 potassium oxalate solution for 4 to 6 hours the weight increases, the anterior tissues gaining most. In M/1000 calcium chloride solution the anterior gain slightly, but no appreciable change occurred in the middle and posterior tissues. Calcium precipitants, hypotonic solutions and lipoid solvents increase, while calcium prevents water appropriation, but the amount imbibed and the degree of protection varies with the region from which the tissue is taken.

62. SOME EFFECTS OF STRYCHNINE ON RECONSTITUTION IN EUPLANARIA DOROTOCEPHALA. Faith Stone Miller, The University of Chicago. (Introduced by C. M. Child.) (15 min.)

Observations were made on the reactions of cut pieces and of intact individuals of Euplanaria dorotocephala to a wide range of concentrations of strychnine sulphate. Treated worms show greatly increased activity, spasmodic contractions, poorly coordinated locomotion, and the reversal of inhibition of antagonistic muscles which is characteristic of strychnine action on annelids and arthropods. They do not exhibit feeding, rheotactic, or righting reactions and they are easily dislodged from the substratum. Strychnine also reduced the fission rate.

Reconstitution experiments on short pieces show greatly increased head frequency at all body levels. Large numbers of bipolar forms appeared in strychnine in the region of the fission zone and to a lesser degree anterior to the pharynx. Many tailless forms which appeared are believed to represent an intermediate condition between head and tail development at the posterior cut surface. With delay of anterior or posterior section head frequencies in strychnine were high at all intervals.

Counts of rates of appearance of eye spots in long pieces from four levels of the anterior zooid were made to determine whether there was any acceleration of development that would account for the great increases in head frequency. The only effect was a very slight delay.

The high head frequency, the large number of bipolar forms, and the lack of acceleration of development lead to the conclusion that strychnine causes decrease or elimination of correlative factors within the piece rather than stimulation of the head forming cells.

63. STUDIES ON HETEROPLASTIC TRANSPLANTATION IN TRICLADS. James A. Miller, Ohio University. (Introduced by Carl R. Moore.) (15 min.)

With ice as anesthetic 338 heteroplastic transplants of the ganglionic region were made between Euplanaria dorotocephala and E. tigrina in the head, pharyngeal region and tail. All possible combinations of dorsal and ventral with anterior and posterior surfaces were used.

An anteroposterior gradient in host resistance to reorganization by the graft was observed. Implants in the head generally produced little host reorganization and were often incorporated. Grafts in reversed anteroposterior orientation often induced development of supernumerary photoreceptors but only those with a free surface induced postcephalic outgrowths.

In the pharyngeal region cephalic grafts caused the regenerating host pharynx to appear farther posterior than in controls. In these a postcephalic outgrowth developed even with complete union, but there was little graft dominance anterior to the implantation level. Grafts in this level tended to migrate anteriorly, particularly during starvation.

Cephalic grafts in tail regions reorganized the host anterior and posterior to implant. Graft dominance anterior to level of implantation extended about to the fission zone. Effects of level of implantation were strikingly demonstrated when grafts were placed in two levels of the same host.

In all levels a free surface was necessary for regeneration by the transplant and determined its anteroposterior axis. In no case was the dorsoventral axis modified. Union of digestive tract of implant and host was necessary for maintenance but not for regeneration of the graft. Long continued observations showed that graft ectoderm was gradually replaced by host tissue.

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Embryology

64. TRANSPLANTATION OF THE POLAR LOBE IN SABELLARIA VULGARIS.

Alex B. Novikoff, Columbia University. (Introduced by L. G. Barth.) (Lantern; 7½ min.)

The normal larva of Sabellaria vulgaris early develops a long apical tuft and later a post-trochal region from which extend two groups of setae. Isolation experiments indicate that the formation of the apical tuft requires materials present in the polar lobe and later to be found in the C cell. Similarly the post-trochal region depends upon polar lobe materials, later present in the D cell.

Transplantation of the polar lobe to two- and four-cell stages and to AB blastomeres showed no induction of apical tufts, the host blastomeres undergoing normal self-differentiation. Transplants were made using cells which receive all or part of the materials of the polar lobe, namely, the CD, C, and D blastomeres. Transplants were made to complete embryos in the two- and four-cell stages, to embryos from which the polar lobe had been removed, and to AB cells. The presence of these cells did not alter the self-differentiation of the host blastomeres. The number of apical tufts which differentiated was the same as the number of C cells present, the number of post-trochal regions the same as the number of D cells.

Thus there seems to be no diffusion from one cell to another of substances leading to the formation of the apical tuft or post-trochal region. The polar lobe cannot therefore be considered an embryonic organizer, at least in the sense of Spemann.

65. MICROCAUTERY OF POSTERIOR POLE-PLASM AND GERM-CELL DETERMINANTS IN DROSOPHILA MELANOGASTER. Joseph W. Jailer, Washington Square College, New York University. (Introduced by R. B. Howland.) (Opaque projection; 5 min.)

Timed eggs of Drosophila melanogaster in which the cleavage nuclei have not yet reached the posterior pole-plasm were dechorionated and aligned on thin slabs of agar. The posterior region, subjected to heat from the point of an electromicrocauter, was burned to an extent varying from one-eighth to one-sixth of the entire egg. The living portion of the eggs constricts off gradually from the burned mass. A blastoderm is laid down around the entire living portion. No germ cells form at the posterior pole. The operated embryos undergo the typical proctodeal-amniotic invagination, but no pole cells are carried inward in this region. From a large number of embryos but one adult hatched. This fly, a male, was crossed to four virgin females. All these females laid sterile eggs. In sections of the small mass of testicular tissue of the male no spermatogonial cells or sperms were seen. It is apparent that there is in the prelocalized posterior egg region, either in the pole-plasm or the granular inclusions or both, the essential materials for germ cell formation.

66. THE DEVELOPMENT AND METAMORPHOSIS OF THE ASCIDIAN PHALLUSIA NIGRA. Oscar W. Richards, Yale University. (Motion picture; 15 min.)

Cleavage, embryo formation, hatching and metamorphosis will be illustrated by a motion picture film taken at the Tortugas Laboratory of the Carnegie Institution. The film record will be used for the measurement of the early growth and changes in the form of this animal.

67. REGENERATION AND DIFFERENTIATION IN CRUSTACEA. Hugh H. Darby, College of Physicians and Surgeons, Columbia University. (Lantern; 15 min.)

Further analysis of the change that takes place when the simple biramous appendage (pinch-claw) of Crangon armillatus becomes modified into a snap-claw yields the following results. If the snap-claw is removed and at the same time a small piece of the dactylus of the remaining pinch-claw is removed, the usual change of pinch-claw to snap-claw takes place at the next molt. However, the piece amputated from the pinch-claw is now missing from the dactylus of the snap-claw. The conclusion is clear that each part of the simple pinch-claw is modified into the corresponding part of the snap-claw.

By varying the size of the piece of dactylus removed from the pinch-claw, the following variations in growth have been induced. When only a small piece is removed, the length of the propodus of the new snap-claw closely approximates that of a normal snap-claw propodus. However, when almost all of the dactylus is amputated, the new propodus is much longer than normal. This shows that at the tip of a growing claw there is competition between dactylus and propodus for growth substrates. A similar competition has already been shown to operate between the two claws. A general competition between parts is suggested.

68. GROWTH OF THE CHICK EMBRYO. T. C. Byerly, W. G. Helsal and J. P. Quinn, Bureau of Animal Industry. (Lantern; 15 min.)

Weights of embryos from Silkie and Single-comb Rhode Island Red matings and from the reciprocal matings between those breeds were obtained for incubation ages of 3 to 20 days, inclusive. Approximate average adult weights for the birds used were: Silkie males, 3 pounds; Silkie females, 2 pounds; Red males, 6.5 pounds; Red females, 5.5 pounds. Approximate average egg weight for Reds, 60 gm.; for Silkies, 40 gm.

Embryos of 11 to 19 days incubation in eggs from Silkie females were smaller than embryos of like age from eggs of Red females. Genetic differences in embryo size were small and only appeared after the first week of incubation. Cell counts of neural tube at the level of the otic vesicle in 48-hour embryos, and of liver in 14- and 18-day embryos, from the stated matings and from Single-comb White Leghorn matings, showed no significant differences in cell size. Genetic differences in embryo size cannot, therefore, be due to inherent differences in the rate of cell division.

69. X-RAY STUDIES IN THE GENETICS AND MORPHOGENESIS OF DROSOPHILA MELANOGASTER. C. P. Haskins and E. V. Enzmann, Harvard University. (Introduced by T. J. B. Stier.) (Lantern; 7 min.)

Experiments are described involving the determination of a 'sensitive volume' for the locus of the white-eye mutation in Drosophila melanogaster. Embryos and young larvae of eosin stock are x-rayed when between 18 and 24 hours of age. It is found that a proportion of the treated individuals shows when adult patches of whitened ommatidia in the compound eye constituting colonies of cells descendant from individual affected cells of the optic anlage. A plot of percentage of affected eyes against dosage is consistently linear, which permits a calculation of the minimum limiting sensitive volume for the total number of loci for white in the eye anlage at the time of irradiation, by the Curie probability method. From this is derived the sensitive volume for the locus in a single cell, and, assuming sphericity, its radius. The radius is found to be in the range of 8×10^{-7} cm., in fairly good accord with other determinations of genic size, and with determinations of Svedberg, Sjogran, Northrop, Kunitz, and others of the radii of various protein molecules.

A discussion is then given of the bearing of this work on studies of the morphogenesis of the compound eye in Drosophila.

70. EFFECTS OF ROENTGEN RADIATION ON EMBRYONIC ORGANIZATION AND REGULATION IN MELANOPLUS DIFFERENTIALIS (ORTHOPTERA). Titus Carr Evans, University of Iowa and A. and M. College of Texas. (Lantern; 7 min.)

Eggs of the grasshopper have been irradiated at ages of 1 to 14 days. It was found that irradiation of eggs of from 6 to 8 days of age (25°C.) resulted in many duplications of parts, and aberrant growths from the head. Some of the outgrowths from the head were extensive enough to show the tendency to form one or more secondary embryos. The developmental stage which yielded the most frequent aberrations following irradiation is comparable to gastrulation which has likewise been found to be susceptible in vertebrates and in other insects by operative techniques. This stage also marks the change from the regulative to the mosaic phase of development in this organism. It has been concluded that the most obvious effect of irradiation, selective injury to more active cells, might account for the abnormalities by acting as a physiological and mechanical block to the critical and extensive processes of organization and differentiation taking place at this time.

71. USE OF ROENTGEN RADIATION IN ANALYSIS OF METABOLISM IN EGGS OF MELANOPLUS DIFFERENTIALIS. Titus Carr Evans, University of Iowa and A. and M. College of Texas. (Lantern; 8 min.)

Irradiation of the grasshopper eggs during diapause does not consistently affect the rate of oxygen consumption until later when the embryo begins developmental activity. It is possible, by raying certain regions of the egg during pre-diapause development at appropriate stages, to produce eggs which will have one or more parts missing at the time of diapause. The rate of oxygen consumption during diapause is not appreciably affected by absence of a) embryo, b) embryonic membranes, c) cuticle, and d) vitellophages.

During pre-diapause development the increases in rates of oxygen consumption and intake of water appear to be dependent on the formation of the embryo. Also, further intake of water and increase in respiration is checked when the embryo is destroyed by irradiation. The last 3 or 4 days of pre-diapause development of the embryo may be inhibited without immediately affecting the water intake or respiration of the egg.

Extremely heavy irradiation (over 3000 roentgens) of the yolk has retarded embryonic development, but when the diapause stage is finally reached the rate of oxygen consumption is higher than that of the controls. It is evident that the apparent depression of respiration in developing eggs is not a direct action on the respiratory mechanism but is simply an inhibition of growth of more respiring material.

72. THE SUSCEPTIBILITY OF FUNDULUS HETEROCLITUS EMBRYOS TO X-RADIATION. Archie N. Solberg, Columbia University. (Introduced by L. G. Barth.) (Lantern; 7½ min.)

One hundred and ten stages in the early development of Fundulus heteroclitus, at 25°C., have been photographed, in order to determine accurately the time interval between stages in its development. The susceptibility of embryos has been tested with four doses (2000 R., 4000 R., 10,000 R. and 20,000 R.) of x-rays, and the subsequent development of embryos followed.

The early cleavage stages show a high sensitivity and when treated with 2000 R. will produce nearly 100% abnormal embryos. With the same dose gastrulating embryos show practically no sensitivity, but a slight rise in sensitivity occurs during the early differentiation of the somites. As embryos develop they become increasingly resistant and after 132 hours can withstand a dose of 20,000 R.

With large doses (10,000 R. and 20,000 R.) 100% abnormalities can be produced by radiating during the early stages, and at any one stage all of the abnormalities will be alike. Differences in sensitivity to a single dose, at different stages can still be demonstrated by the extent of differentiation of the anterior-posterior axis of the embryos.

The injurious effects of radiation seem to be primarily on cell division which prevents proper growth, inductions, and differentiation. The latent period before visible effects appear depends 1) on the stage in development, and 2) on the dose of the radiation.

73. X-RADIATION AND THYROID-INDUCED METAMORPHOSIS IN ANURAN LARVAE.

William O. Puckett, Princeton University. (Introduced by C. F. W. McClure.) (Lantern; 15 min.)

A precocious metamorphosis of Rana sylvatica tadpoles has been obtained by a single feeding of desiccated thyroid substance. Metamorphosis is complete 8 to 10 days after the single thyroid feeding. Tadpoles exposed to non-lethal dosages of x-radiation (700 r) 6 days previous to thyroid feeding react quite differently from control tadpoles. They show a heightened sensitivity to thyroid treatment during the early or regressive phases of metamorphosis, completing these stages much more quickly than control animals. However, they are totally powerless to complete the later stages of metamorphosis involving the differentiation of structures characteristic of the frog.

Radiation of a portion of the tadpole body followed by thyroid treatment results in a partial metamorphosis. Regions of the body screened from the x-radiation attain a complete metamorphosis while exposed portions are unable to perfect the differentiative phases of metamorphosis. This suggests a purely local effect of x-radiation.

Dissections and histological studies show that those tissues most active metabolically at the time of metamorphosis, namely, the tissues of the alimentary tract, are most severely affected by the x-radiation. The gut undergoes a complete dedifferentiation and suffers a normal reduction in length, but the cells thus produced are unable to organize themselves into the structures characteristic of the alimentary tract of the frog.

74. THE EFFECT OF LIGHT AND TEMPERATURE ON THE GONADS OF LEBISTES.

Glenn C. Dildine, Northwestern University. (Lantern; 10 min.)

Three groups of mature Lebistes of both sexes were kept at 18°C., 23°C. and 28°C., respectively. Temperatures of 18° and 28° are close to the survival limits of the fish. 1) Data from females show the importance of temperature in brood production. Eight females kept at 18° produced an average of 1.62 broods each during a 100-day period; nine females kept at 23° averaged 2.67 broods each during the same period. During 78 days, three females kept at 28° averaged 1.33 broods each. There was no significant difference between animals kept darkened and those illuminated with ordinary electric bulbs. 2) Data from males are based on histological examination of the testes of sixteen specimens kept under the experimental conditions for 60 days (seven at 18°, seven at 23°, and two at 28°). The proportion of various maturation stages in the testes shows no constant differences between the 18° and the 23° groups; more animals are being tested to check against the apparent predominance of late maturation stages in the two 28° animals.

75. THE OVARIAN CYCLE OF THE VIVIPAROUS TELEOST, SKIFFIA BILINEATA, A MEMBER OF THE FAMILY GOODEIDAE. Guillermo Mendoza, Northwestern University. (Introduced by C. L. Turner.) (Lantern; 15 min.)

In Skiffia bilineata, the ovary produces the ovocytes and harbors the developing embryos until time of birth. Fertilization occurs while the ova are within the ovigerous tissue; evacuation into the ovarian lumen follows immediately. The developing embryos are there retained until birth. Since the embryos possess but little yolk, the ovary furnishes a nutrient and respiratory medium which bathes the embryos.

Cyclical changes in the somatic tissue involve mitotic activity, types and numbers of free cellular elements, and secretory activity. Shortly after fertilization mitotic activity of the epithelium increases rapidly and is followed by a rise in secretory activity. Both mitosis and secretion then declines slowly until the young are expelled. Free cellular elements, some of which are concerned with secretion, are especially numerous up to the middle stages of gestation at which time a maximum is reached and after which there is a decrease until birth of the young. During the middle stages of gestation, in addition to the secretory activity of the epithelium, the folds become turgid with fluid.

Production of ova is high at time of fertilization and gradually decreases as the young grow; however, in the latest stages of gestation, a marked increase in production of ova is again evident. Degenerating ova are more numerous at this late stage, conforming with known facts of egg production.

These marked changes in germ and soma fluctuate during the breeding season with the production of broods approximately every 46 days.

76. THE REPRODUCTIVE CYCLE IN THE MALES OF SOME CAVE BATS. Roland E. Miller, University of Missouri. (Introduced by Mary J. Guthrie.) (Lantern; 12 min.)

Testes, epididymides and accessory glands have been studied in two species of cave bats, Myotis lucifugus and Myotis grisescens, collected throughout the year. During the hibernating period from November to April the testes exhibit no spermatogenetic activity, the epididymides are packed with spermatozoa, and the accessory glands are large and filled with secretion. Occasional mitotic figures occur in the basal layer of the germinal epithelium in the seminiferous tubules throughout this period. In early June the testes are enlarged, and the nuclei of the primary spermatocytes are in the prophase (synaptic stages) of the first meiotic division. The epididymides are very small and do not contain spermatozoa. The accessory glands contain no secretion and are reduced to a condition similar to that found in immature animals. In July meiotic figures first appear. Sperm heads in the testes have been observed first in mid-July. Motile spermatozoa appear in the epididymides in August. By September the epididymides are distended with spermatozoa, and the accessory glands are filled with secretion and extremely hypertrophied. In October, the period of fall copulation, the testes are again in an inactive condition and there is a slight reduction in the number of spermatozoa in the epididymides. The accessory glands are somewhat reduced in size to a level of hypertrophy which is maintained until late spring. A study of the interstitial cells is in progress.

Cytology

77. EXPERIMENTAL MODIFICATION OF CHROMOSOME BEHAVIOR DURING REDUCTION DIVISIONS IN THE EGG OF NASSA (ILYANASSA). G. B. Moment, Goucher College. (Lantern; 7 min.)

The eggs of this snail have proved favorable material for such studies due to the fact that the time interval between laying and the emergence of the first polar body and between that event and the second polar body is relatively long. According to the precosity theory of the cause of meiosis, lengthening the time between successive divisions might be expected to convert meiotic into somatic mitosis. In these experiments eggs were anesthetized after the extrusion of the first polar body with the minimal concentration of chloral hydrate to completely inhibit polar body formation and were then returned to sea water at various intervals. After approximately an hour either a single second polocyte several times the usual size is formed or else two (or in very rare cases three) 'second' polar bodies emerge simultaneously from the animal region of the egg. They are usually separated from each other by a space greater than their own diameter. Feulgen's stain clearly reveals chromosomes in them. Subsequent cleavages so far have been irregular.

78. THE FREQUENCY OF RECIPROCAL TRANSLOCATIONS IN IRRADIATED GERM CELLS OF CIRCOTETTIX VERRUCULATUS (ORTHOPTERA). Edwin R. Helwig, University of Pennsylvania. (Lantern; 15 min.)

Grasshoppers were irradiated just prior to hatching at which time only primary spermatogonia are present in the gonads. Each primary spermatogonium is the progenitor of all the cells within a single cyst so that any chromatin rearrangements produced in such a spermatogonium will be present in every cell within that cyst. Seventy-three cysts in which translocations were found in every cell were studied. Sixty-five of these contained one or more closed-circle multiples, thus indicating that reciprocal translocations had occurred. Eight cysts had no cells in which closed-circle multiples could be found but chain multiples were present in all of them. Due to the configurations of the multiple chromosomes in the spermatocytes of three of these cysts it was clear that true translocations had not occurred but that two entire chromosomes had fused at their proximal ends. The mode of synapsis of the chromosomes in some of the cells of four other cysts indicated that reciprocal translocations were responsible for the configurations obtained. All the spermatocytes of the remaining cyst contained chain multiples of such a shape that only a single translocation was indicated. However, this case is unsatisfactory because one of the chromosomes involved was so small after the greater part of it had become translocated that it rarely entered into any synaptic union. Thus, the evidence would indicate that single translocations are of infrequent occurrence if they occur at all.

79. MULTIPLE CHROMOSOME COMPLEXES IN THE METAMORPHOSIS OF CULEX PIPIENS. C. A. Berger, Johns Hopkins University. (Introduced by C. W. Metz.) (Lantern; 8 min.)

Multiple complexes of ninety-six, forty-eight, twenty-four and twelve chromosomes, and the normal diploid number of six were found in epithelial cells of the pupal ileum during metamorphosis. In larvae the ilial epithelium grows by increase in cell size, not multiplication. When larva hatches the nuclei have an average diameter of from 3 and 4 μ. These same nuclei increase greatly in size and become flattened. Greatest diameters range from 10 to 17 μ. This epithelium does not undergo disintegration. New tissue is composed of a larger number of smaller cells. Apparently each larval cell undergoes three or four divisions during metamorphosis.

The first divisions always show higher multiple complexes. Later, cells and complexes of all sizes are present. Toward the end of metamorphosis smaller cells and complexes of twelve or six are abundant. In prophase three pairs of long thick threads condense into three pairs of short thick rods each composed of eight or more elements. Each of these six groups now falls apart into its constituent units. These unite as bivalents and move to the metaphase plate. Early anaphases suggest that chromosomes do not split but that bivalents separate into their constituents. (Somatic reduction.) By the time the chromosomes reach the poles they have again united to form bivalents.

This multiplication of chromosomes during the long larval resting stage and the subsequent series of somatic reduction divisions with short intervening interphases may be a means of effecting rapid metamorphosis of this tissue.

80. STUDIES ON SALIVARY GLAND CHROMOSOMES IN DIPTERA. C. W. Metz, Carnegie Institution of Washington. (Lantern; 7 min.)

Studies deal with 1) nature and interrelationships of 'discs,' 'bands,' 'granules,' 'vesicles' and 'capsules' and 2) possible significance of knots in relation to structural rearrangements and mutation.

Evidence indicates that in many, if not most, cases a transverse plate ('band') of granules includes material from two or more chromatic 'discs'; also that one disc may contribute to two separate transverse rows (bands) of granules. This adds further uncertainty to the already doubtful significance of the 'granules.' Although the bands seem definitely to have significance as approximate indicators of gene loci, there seems to be no valid reason for regarding each single granule as in any sense representing a single unit gene.

'Capsules' or 'vesicles' appear in definite positions—not at random. They are apparently formed by peripheral convergence of two adjacent discs, like two saucers face to face. Behavior indicates qualitative difference between achromatic materials in different regions of chromosome.

Not infrequently, in untreated salivary glands of Sciara, a chromosome (pair) is tied in a true and 'tight' knot. Assuming that such knots occur in cells of the germ-line, they provide an interesting possible mechanism for chromosome rearrangements. With its three points of overlap each knot could result in any one of various kinds or combinations of inversions and deletions. Such knots also provide further suggestion of the presence of an insulating layer of 'matrix' or 'sheath' material which may be broken down artificially (irradiation, etc.) to permit chromosome contacts resulting in rearrangements and possibly 'gene mutations.'

81. THE DEPENDENCE OF NUCLEAR COLLOIDAL ORGANIZATION UPON THE CATION RATIOS OF A RINGER MEDIUM. William R. Duryee, Columbia University. (Lantern; 15 min.)

Nuclei (germinal vesicles), when isolated from ovarian eggs of Rana temporaria, and from six other species of European amphibians and teleosts, contain at least four concentrically arranged colloidal systems: a) the main nuclear matrix, b) the three types of diffuse nucleoli, c) the inner nuclear core (anlagen of first polar spindle) in which the chromosomes are embedded, and d) the thirteen pairs of chromosomes. This normal arrangement is maintained by specific ratios of the Na-K-Ca components of the medium.

In NaCl 0.01 to 0.025 M. the nuclear volume increases 200 to 600% and the nuclei become microscopically transparent, while above the critical concentration of 0.04 M. swelling is less and the nucleoli no longer dissolve. But the principal antagonism with regard to swelling and clearing as against shrinking and flocculation is between K and Ca in approximately their concentrations in amphibian Ringer. Na in similar concentrations appears to 'protect' the nuclear colloids against the violent coagulative effect of Ca. Transparent chromosome pairs, which never dissolve in the strict sense, can be progressively and quantitatively precipitated from their also transparent matrices by Ca, Mg, Hg, Ag, basic dyes, or certain acids, and perhaps by asphyxia. When $CaCl_2$ approx. 0.001 M. or less is added with great care the process appears reversible.

82. AN ATYPICAL MEIOTIC DIVISION IN THE PARASITIC WASP NEMERITIS. B. R. Speicher, Columbia University. (Introduced by P. W. Whiting.) (Lantern; 10 min.)

In Nemeritis canescens Grav., which reproduces by parthenogenesis, all offspring are diploid and female. Oogonial and cleavage metaphases show twenty-two chromosomes. The first meiotic division exhibits normal pseudo-reduction to eleven tetrads, followed by partial fusion of all tetrads into a single mass, a stage corresponding to early anaphase and possibly caused by imperfect fixation. Slightly later in anaphase the mass separates into two groups, each with eleven dyads, moving to opposite poles. The dyads are at first taut with the stress of anaphase movement, but soon become relaxed and anaphase movement ceases. The direction of chromosome movement is then reversed so that both anaphase groups return to a common metaphase of twenty-two dyads. Homologous dyads do not synapse in this second metaphase which is distinct both by appearance and by time of occurrence from the first. The second anaphase is probably an exact duplication of the first as regards chromosome segregation and again shows eleven dyads moving to each pole. Thus the result of two meiotic divisions is a single polar nucleus and an egg nucleus which after a brief interphase forms a first cleavage metaphase with the diploid number of chromosomes.

83. The physiological properties of the rabbit oocyte and its sheaths. G. S. de Renyi and D. P. Murphy, University of Pennsylvania. (Introduced by S. A. Matthews.) (Lantern; 15 min.)

Ovulation in the rabbit was induced by intravenous injection of pregnancy urine (Friedman's method). The oocytes were washed out from the oviducts shortly after ovulation. The physical properties of these oocytes and of their sheaths were studied with microdissecting and microinjecting methods. It was found that the cells of the cumulus are held together by a highly viscid intercellular substance. The zona pellucida is an extremely rigid membrane with almost no elastic properties. The oolemma has quite a high refractive index and resists disintegration after injury. The ooplasma and the nucleus are of a soft, gelatinous consistency.

84. Polar protoplasmic behavior during cell division. W. J. Baumgartner, University of Kansas. (Lantern; 15 min.)

The protoplasm of living germ cells around the polar areas during the late anaphasic and telophasic stages shows some very peculiar behaviors. The cell wall apparently becomes thinner and is less resistant; long pseudopodia-like processes project out from the poles. At other times large bulges or bubbles extend out, or deep depressions occur in the walls. The pseudopodia or bulges are often withdrawn and others projected bringing about relatively active movement at the poles. The broadness of the pseudopods or the size of the bubbles changes from time to time in a cell, or from cell to cell in succeeding set-ups. The time of onset, the size of the projections and the extent of the movements depends partly upon the hypotonicity of the nutrient solution used—the greater the salt content the greater the effect. After the separation of the daughter cells, the pseudopods are withdrawn and the walls thicken and the cell rounds up. Sometimes small parts of protoplasm are left outside the cell. Non-dividing cells or the sides of dividing cells do not show pseudopods unless the hypertonicity becomes too great. An explanation of this behavior is likely to be found in the flowing of cytoplasm toward the poles during cell division.

85. Distribution of minerals in the cells of some human tissues. Gordon H. Scott, Washington University School of Medicine. (Introduced by E. V. Cowdry.) (Lantern; 15 min.)

A fortunate circumstance permitted the collection of a number of human tissues within ½ hour after death. These specimens were prepared for microincineration by the Altmann-Gersh frozen dehydration method and by fixation in alcohol and formalin. Some remarks will be made concerning the fixation and the tissues from each fixation compared. The results obtained will be presented and discussed. Particular reference will be made to the nuclear minerals in both resting and dividing cells.

86. The cytological changes produced in the basophilic cells of the hypophysis by various treatments of the testes. Arthur J. Gatz, Carleton College. (Introduced by D. E. Minnich.) (Lantern; 15 min.)

In making this cytological study of the basophilic cells of the albino rat hypophysis, it was necessary to observe the histological effects of artificial cryptorchidism, x-radiation, vasectomy, and of senility on the testes. The germinal epithelium exhibited a degenerate condition in all of the experiments (except following castration, after which it was absent). The interstitial tissue of the testes remained in the normal condition but often appeared hypertrophied.

The basophils of the anterior lobe of the hypophysis exhibited a progressive hypertrophy following the loss of the seminiferous epithelium. This hypertrophy affected the cytosome, the nucleus, and the Golgi material. The number of the basophils increased markedly following the loss of the germinal epithelium. The basophils may be arbitrarily separated into three groups:

a) Smaller homogeneous cells which have the appearance of the basophils in the normal hypophysis. These cells, apparently, have recently differentiated from the chromophobes.

b) Large cells which show numerous small osmiophilic granules scattered in the cytosome and possess Golgi material which is much larger than that found in the normal animal.

c) Large vacuolated cells, which have originated from the basophilic cells and termed the 'sterility cell' since the cell appears 2 months following any degeneration of the germinal epithelium. The Golgi material often was not found as a distinct body, but took the form of discrete granular or thread-like particles. The vacuole contains a finely granular substance—which is thought to be the gonadotropic hormone of the hypophysis. This cell is considered to be a storage cell.

87. A study of the bacterial and alleged mitochondrial content of the cells of the clover nodule. E. DeWitt Miller, University of Virginia. (Lantern; 15 min.)

Mitochondrial techniques, employed in the study of cell inclusions in the nodules of clover, reveal many minute forms, both rod-like and granular, which have physico-chemical properties of both bacteria and mitochondria. Following prolonged treatment of nodules with such lipoid solvents as 95% alcohol and ether prior to fixation by recognized mitochondrial methods, many minute rods and granules persist which color red with anilin-fuchsin and methyl green or toluidin blue. Similar inclusions have been observed in nodules after fixation with Altmann's fluid containing 5% acetic acid. Halves of the same nodule which had received different treatment prior to fixation, then sectioned and mounted side by side upon a slide for comparative study, showed in many cases no observable differences in number and nature of minute cell inclusions.

Dry smears of nodules, cultures on artificial media of bacteria from the nodule and from the soil, following mitochondrial methods of fixation and staining, revealed minute bacterial forms both rod-like and granular which in morphologic and tinctorial properties were indistinguishable from inclusions occurring in the nodule and formerly believed to be mitochondria.

Endocrinology

88. THE PHYSICAL BASIS OF ACCELERATED BARB FORMATION BY FEMALE HORMONE AND THYROXIN IN CERTAIN FEATHER FOLLICLES. Richard M. Fraps, The University of Chicago. (Introduced by Frank R. Lillie.) (Lantern; 15 min.)

Either female hormone or thyroxin in sufficient concentrations increases the frequency of barb formation per unit axial growth in certain feathers—notably those of back and saddle tracts—of the Brown Leghorn capon. These and other quantitative relations are characteristically different under action of the two hormones. The acceleration of barb formation by thyroxin is of particular interest in view of the rapidity of action and marked variability of effect at different levels of comparable feathers. Evidence is presented in support of the conclusion that frequency of barb formation and number of barbs laid down under the action of thyroxin is determined largely by extent of the undifferentiated ventral field. Thyroxin effects rapid differentiation of this ventral region; it does not appear to cause growth of tissue which subsequently differentiates as barb primordia. The ventral field is of greater extent at distal barb levels, and correspondingly, barb frequencies are increased to greater degree in these cross sections than at lower levels. The absence of marked increase in barb frequency in feathers from other tracts of the Brown Leghorn is correlated with limited ventral fields. Similar considerations determine the range of female hormone in acceleration of barb formation.

89. VARIATION IN SUCCESSIVELY REGENERATED FEATHERS FROM SINGLE FOLLICLES OF HYBRID FOWL AND THEIR REACTION TO FEMALE HORMONE. (F_1, BARRED ROCK ♂-BROWN LEGHORN ♀, AND RECIPROCAL MATING.) Mary Juhn, The University of Chicago. (Lantern; 15 min.)

The F_1 males are barred but with numerous exceptional leghorn-like feathers in the lacy plumage areas. In the saddle the exceptional feathers most frequently have a barred distal section and a leghorn base. Barred and leghorn components are simultaneously present in the formative zone, the collar, of the feather germ. The transition of barred to leghorn may occur in two adjacent barbs but in the great majority of cases observed the apical barb segments continue barred while the basal barb segments become leghorn.

Records for three generations of feathers grown from single follicles have been published for a number of individuals; barred feathers are succeeded by exceptional feathers and vice versa but there is a greater tendency toward barring. Successive exceptional feathers from a given follicle do not correspond in pattern. A similar situation obtains in five successive regenerations of feathers from single follicles. Somatic mutations cannot explain these results.

Female hormone injections commenced shortly after plucking cause regeneration of female barred feathers. Exceptional feathers grown into the leghorn phase before female hormone injections are commenced become modified to female shape and structure but they are metallic green in color. Occasionally barring extends further basally in one vane-half of exceptional feathers and female hormone injections give rise to female feather segments barred in one vane-half and metallic green in the other.

90. FURTHER EXPERIMENTS ON THE THYROTROPIC FIELD AROUND THE IMMATURE TADPOLE'S PITUITARY. William Etkin, City College and American Museum of Natural History. (Lantern; 12 min.)

Thyroid primordia were transplanted to the region of the pituitary primordium in tail-bud embryos of Rana pipiens and R. sylvatica. Similarly pituitary primordia were transplanted to the thyroid region.

The physical approximation of the glands by either method yielded essentially the same result, metamorphic precocity. The number of animals showing precocity in different experiments varied from six out of thirty to forty of forty-four. This precocity took the following forms. In pipiens hind leg growth became very rapid at about 20 mm. total length of the host. In sylvatica this effect appeared slightly but not proportionately earlier. The subsequent metamorphic picture varied. In pipiens hind leg growth generally continued rapidly. Sometimes later metamorphic events took place precociously and rapidly, but in most cases a metamorphic stasis appeared about the stage of beak resorption. In sylvatica the usual picture was one of extreme precocity in later metamorphic events but with apparent stasis before metamorphosis was quite complete, or sometimes earlier.

Histological findings. In pituitary graft hosts killed early in precocious metamorphosis a very much enlarged and partially or highly activated thyroid was found. In thyroid graft hosts, the graft was as above but the normal-site gland was small and inactive as in controls. In animals sacrificed later in metamorphosis the histological findings varied, most indications were of a decrease in activity compared to the earlier stages.

91. RELATION OF THE ADRENAL CORTEX TO HAIR GROWTH IN THE WHITE RAT AS SHOWN BY REMOVAL AND TRANSPLANTATION EXPERIMENTS. Earl O. Butcher, Hamilton College. (Lantern; 10 min.)

The second growth of hair appears externally on the dorsum of the white rat about the thirty-sixth day. When bilateral adrenalectomy is performed on the twenty-third day, the second growth of hair occurs 4 or 5 days earlier. Not only does the hair in the adrenalectomized rat grow sooner but it also has a better quality. Evidence that this precocity occurs in the absence of the cortical portion of the adrenal is furnished by the following: 1) death occurs frequently from cortical deficiency after the growth is well started; 2) if the adrenals are removed and transplanted into the kidney, the medulla disappears; when the cortical portion persists, hair growth is normal; 3) and those animals that survive the adrenalectomy, having had a precocious growth in the second cycle, have a normal growth in the third cycle. This survival and third growth are undoubtedly due to the hypertrophy of extra cortical bodies. The absence of the medulla is apparently not responsible for the precociousness of the second cycle or a similar acceleration would occur in the third growth. Further investigations are being conducted to determine how the decrease of cortical material accelerates the hair growth.

92. ADRENAL INSUFFICIENCY. A STUDY IN GENERAL PHYSIOLOGY. I. BLOOD OSMOTIC PRESSURE. E. W. Blanchard, Bryn Mawr College and Biological Laboratory, Cold Spring Harbor. (Lantern; 10 min.)

With few exceptions it has been generally accepted that the principal function of the adrenal cortical hormone is to control, in a manner not completely understood, the electrolyte and water regulation of the blood and tissues. Other work coming from this laboratory indicates that a change in blood lipid partition occurs following bilateral adrenalectomy; this alteration being a marked shift in lipid from plasma to cell. The relation of this change to possible modifications of cell permeability has been suggested.

In this experiment the osmotic pressure of the blood serum was followed by the freezing point method of Johlin in normal, unilaterally operated, and bilaterally adrenalectomized cats. Following the removal of the second gland a gradual and marked drop in serum osmotic pressure occurs as evidenced by a decrease in the freezing point depression of the serum. This change is not a terminal one, but follows closely upon the second operation.

If some factor such as an altered cell membrane permeability resulting in a loss of electrolyte from the blood follows bilateral adrenalectomy the tonicity of the blood would fall, water would enter the tissues, and, upon the establishment of equilibrium, a relative decrease in the plasma volume would occur, the smaller amount of plasma, nevertheless, having a decreased tonicity. Such an alteration in tonicity does follow bilateral adrenalectomy and lipid shift may well account for the altered permeability.

93. THE HISTOLOGY OF THE MAMMARY GLANDS OF ADRENALECTOMIZED LACTATING RATS. Irving Levenstein, Washington Square College, New York University. (Introduced by Harry A. Charipper.) (Lantern; 10 min.)

The litters of adrenalectomized mothers are not able to survive to the weaning time. This is due to a deficiency in the secretory activity of the mammary glands of the mothers. The histological picture of these glands shows only slight structural changes from normal secreting glands. Secretory elements are present and functional. The most striking difference is that the alveoli are less distended and apparently contain a more dense secretion. Although the litters of untreated adrenalectomized rats generally die of starvation, milk secretion does not completely stop as long as the litters are still suckling. The normal histological picture of the mammary glands as well as their function can be, in most cases, largely restored by salt therapy. An analogous condition to that found in the mammary glands of operated animals can be brought about by depriving normal lactating animals of fluids. These results are interpreted as a substantiation of the theory that the deficiencies following adrenalectomy are due largely to the disturbance in water and salt balance.

94. ON THE EFFECTS OF HORMONES OF SPERMATIC FLUID UPON THE OYSTER. T. C. Nelson, Rutgers University. (Lantern; 15 min.)

A hormone in oyster sperm stimulating spawning in male and female oysters was demonstrated by Galtsoff ('30). A second hormone causing increased water pumpage in the male oyster was reported by Nelson ('35). Further research reveals at least four distinct responses caused by fresh oyster sperm: 1) the stimulation of spawning as shown by Galtsoff; 2) immediate relaxation of the adductor muscle permitting the shells to gape to their widest possible extent; 3) enlargement of the ostia due apparently to relaxation of the vertical blood vessels of the gills with resulting construction of the blood vessels of the interfilamentar junctions; 4) immediate increase in the speed of ciliary movement. The last three responses cause rapid increase in the rate of water flow through reducing the friction by opening up the avenues of water passage, and through increasing ciliary movement. These effects reach a maximum in less than a minute after addition of fresh oyster sperm, hence cannot require swallowing of sperm and absorption from the stomach as held by Galtsoff for stimulation of spawning. The hormones causing increased water pumpage are associated with the sperm and filter with difficulty. They are quickly denatured by drying and by petroleum ether, and are precipitated by saturated ammonium sulphate, the precipitate after dialysis retaining much of its original potency. The responses to all these hormone effects show the male to be more sensitive. They are of value to the oyster in aiding group spawning with its increased chances of fertilization.

95. NATURAL DURATION OF LARVAL LIFE IN PHALLUSIA NIGRA. Caswell Grave, Washington University. (Lantern; 15 min.)

A study of the natural duration of the free-swimming periods of larvae of Phallusia nigra was made during the summer of 1936 in order to establish a basis for estimating the effectiveness of methods of experimental treatment that have caused acceleration of metamorphosis in larvae of this species to the extent of 100% within 2 hours after hatching.

Seven lots of larvae varying in numbers between 2178 and 5252 were segregated immediately after hatching in shell vials each containing 10 cc. of sea water and receiving larvae numbering between 78 and 159. The vials, thirty-two to sixty-four in number, were arranged on a table in two, three or four rows of sixteen each. At intervals of 3 hours formalin was added to the vials one row at a time until counts of larvae and zooids per vial showed all larvae metamorphosed.

From these data seven frequency curves were constructed which show that larvae produced early in the breeding season (June 16th) metamorphose after free-swimming periods varying from about 6 to 48 hours while larvae produced late in the season (August 1st) metamorphose after a larval life varying from about 1 to 30 hours. Evidence shows this gradual shortening of the free-swimming period is not due to an ageing of eggs in the oviducts or to any oviducal influence. It is more likely due to a gradual diminution in yolk supply of eggs as egg production increases. Differences in yolk content of eggs cannot, however, account for the great variability of larvae of the same lot in the duration of larval life.

96. CULTURES OF SOME MARINE INVERTEBRATE TISSUES. Ralph Buchsbaum, The University of Chicago. (Lantern; 10 min.)

Surprisingly little is known of the behavior of cells of invertebrates in tissue culture. This is partly because of the difficulty in obtaining tissues free from bacteria, and partly because of the lack of knowledge of suitable culture media. Established methods in vertebrate tissues culture were applied in making cultures of Aurelia mesoglea, Chiton heart, Panulirus heart and blood, Aplysia heart and blood, and others. Tissues, obtained by surgical asepsis, were kept in natural physiological media such as chicken plasma, lobster blood, etc. Abundant migrations of cells were seen, but mitosis was never observed. What has been described by others as amitosis or mitosis is interpreted to be the meeting and then separation of cells in migration. Knowledge of the best physiological conditions for invertebrate tissues must be obtained before generalizations on fundamental problems of invertebrate tissue physiology can be made.

97. CELL SIZE AND GROWTH IN MOSQUITO LARVAE. Wm. Trager, Rockefeller Institute for Medical Research. (Lantern; 10 min.)

Larvae of the yellow fever mosquito, Aëdes aegypti, were reared at 28°C. in a bacteria-free medium. Every day throughout the larval stage a number of the larvae were killed and their body length measured. From sections of these larvae measurements were made of the cells and nuclei of a variety of tissues. It was found that the larval hypoderm, fat body and ganglia grow chiefly by cell multiplication. These tissues persist without histolysis or other radical change into the adult stage. The larval midgut, hindgut, salivary glands, longitudinal abdominal muscles, main tracheal trunk and oenocytes grow mainly by increase in cell size and all these tissues are destroyed during or immediately after the pupal stage. The Malpighian tubules also grow by increase in cell size, but they survive the pupal stage and are apparently functional throughout adult life. This fact constitutes an important exception to the rule, which otherwise holds fairly well, that larval cells which increase in size and become greatly hypertrophied are destroyed during metamorphosis.

Morphology

98. THE NERVOUS SYSTEM OF DOLICHOGLOSSUS KOWALEVSKYI. Walter N. Hess, Hamilton College. (Lantern; 10 min.)

Dolichoglossus has a subepithelial nerve network which lies underneath the entire outer body epithelium. This network is thickened in the mid-dorsal and mid-ventral regions of the collar and trunk forming the dorsal and ventral nerve cords. The dorsal nerve cord of the collar, which extends into the proboscis stalk, is the most highly organized region of the nervous system and apparently is the coordination nervous center of the animal. Giant nerve cells were found here but not elsewhere.

The subepithelial nerve network is composed of bipolar nerve fibers bearing elongated nerve cell bodies which extend in both an antero-posterior and a circular direction. Many of these cell bodies, which are usually very much elongated, stain more densely and are wider at one end than at the other. Similar nerve cell bodies were also found in the dorsal and ventral nerve cords. A multipolar nerve plexus was found in certain internal regions of the body.

99. OBSERVATIONS ON THE ENTERIC NERVE PLEXUS OF THE EARTHWORM, LUMBRICUS TERRESTRIS. Charles G. Rogers, Oberlin College. (Lantern; 8 min.)

In the lining epithelium of the stomach intestine of the earthworm, Lumbricus terrestris, occurs a plexus of small unipolar, bipolar, tripolar and multipolar cells, with the fibers arising from them. Some of these cells are apparently neurosensory; some may be adjustor, some may be motor. This plexus lies in the epithelial layer, near the bases of the epithelial cells, and, so far as our observations go, wholly on the epithelial side of the basement membrane. The cells making up this plexus are more highly developed than the primitive cells comprising the Coelenterate nerve net, and the fibers arising from these cells form small bundles arranged in the form of a loose network. No branching or anastomosing of these fibers has been observed. On the outer (muscular) side of the basement membrane of the pharynx and oesophagus is a plexus composed of somewhat larger nerve cells and fibers. Such a plexus may, and probably does, occur in the stomach intestine region but has not been demonstrated in our preparations.

Physiological studies upon these plexuses are being undertaken.

100. BRANCHIAL REDUPLICATIONS FOUND IN AMBLYSTOMA. William W. Ballard, Dartmouth College. (Introduced by W. B. Unger.) (Lantern; 15 min.)

A pond in Hanover, N. H., has for 3 years yielded Amblystoma jeffersonianum embryos showing many branchial anomalies. These vary from the appearance of a whole extra visceral arch with all its associated organs on both sides, to only a slight overgrowth in the VI (last normal) arch on one side. A bunch of A. punctatum eggs received from North Carolina contained many individuals which developed external gills on the VI arch, which normally carries none. Among many larvae of A. tigrinum raised from eggs collected near Chicago, one such specimen has been found. The anatomy of these overgrowths has been studied.

In the most advanced cases, the cartilages of the VI arch are duplicates of those in the V arch, and the cartilages of the supernumerary (VII) arch are like those of the normal VI. Muscles which normally arise on the VI arch (transversus ventralis IV and subarcualis IV) take origin from VII instead. The subarcualis IV which normally inserts on the proximal ends of the epibranchial cartilages in arches III, IV and V, inserts here also on the epibranchial of VI.

If there is an external gill on the VI arch, it may be very rudimentary or full sized, with levator and depressor branchiae muscles always present in it, innervated by an extra visceral ramus from the vagus. These muscles have been found weakly developed in the VI arch, with vagus innervation, even in the absence of a gill.

If a VI gill is present, the VI aortic arch sends respiratory capillaries into it. If a cartilaginous VII arch is present, there may be a complete extra aortic arch with it, and the pulmonary artery, normally springing from the VI efferent branchial artery, may come partly or entirely from the VII.

These anomalies are discussed in relation to the classic theories of branchial and laryngeal homologies.

101. THE RELATION OF GERM CELL DEGENERATION TO MODIFICATIONS IN THE TESTICULAR STRUCTURE OF PLETHODONTID SALAMANDERS. J. Wendell Burger, Princeton University. (Introduced by E. G. Butler.) (Lantern; 12 min.)

Spermatogenesis in Plethodontid salamanders progresses over the testis in a caudo-cephalic wave. In the annual cycle, non-sterile portions of the testis, located at the anterior and posterior ends, fail to produce spermatozoa. Associated with the presence of these regions in six species studied, are two types of germ cell degeneration. One type, located in the anterior region of the testis, involves many secondary spermatogonia; the other, localized in the posterior end, begins with young primary spermatocytes, and reduces this region to a cord of primary spermatogonia. A zone free of degenerations intervenes between the two regions of degeneration. An abortive spermatogenetic cycle of immature males shows identical degenerations involving the whole testis. Since Eurycea quadridigitata has posterior degenerations only in the summer, these degenerations apparently are not due to the deliterious action of cold weather, as has been suggested. From evidence derived from a comparative study of mature and immature male cycles, and from inanition experiments, it is apparent that more of the testis becomes activated than can be brought to a successful spermatogenesis. It is suggested that the degenerations result as a by-product of the establishment of a functional zone. Their peculiar sequence results from the caudo-cephalic wave of spermatogenesis. As the wave progresses forward the posterior cells are left outside the future functional zone, and hence degenerate. The anterior degenerations mark the inhibition of the anterior progress of spermatogenesis.

Protozoölogy

102. THE EFFECT OF VARIATIONS IN THE LENGTH OF THE INTERENDOMICTIC INTERVAL ON THE LENGTHS OF SUBSEQUENT INTERVALS IN PARAMECIUM AURELIA. T. M. Sonneborn, John Hopkins University. (Charts; 7 min.)

Different parts of a clone may undergo corresponding endomixes at different times, and these differences can be accentuated by experimental means. When such differences in time of endomixis exist at one period of endomixis, they persist, on the average, at the next and at subsequent periods of endomixis. In such experiments, the average interendomictic intervals are approximately the same for all parts of the clone, but the mean dates of endomixis for the different parts of the clone are very different. A similar relation is shown statistically by the coefficient of correlation of 0.003 ± 0.039 between the lengths of the first and second interendomictic intervals in a collection of 294 pairs of successive intervals. There is thus no tendency for the second interval either to increase or to decrease as the first intervals are lengthened. The same result is reached by more detailed analysis of the data: there is neither inheritance of differences in length of the interval nor compensation for intervals of extreme magnitude. Regardless of the date of occurrence of endomixis or the length of the preceding interval, the following interval tends to be of a constant average length.

103. THE RELATION BETWEEN TIME SINCE THE LAST ENDOMIXIS AND THE TENDENCY FOR ENDOMIXIS TO RECUR IN PARAMECIUM AURELIA. T. M. Sonneborn, Johns Hopkins University. (Charts; 8 min.)

The interendomictic interval varies greatly in extent among different lines of a clone of Paramecium aurelia. Calculations based on 1149 interendomictic intervals observed in isolation cultures show that the probability of an endomixis occurring varies with the lapse of time since the last endomixis. The probability is very low when less than 10 days have elapsed, rises abruptly to a high value when 10 to 20 days have elapsed, declines greatly during the next 10 days, and thereafter gradually decreases until it reaches zero after the lapse of about 100 days. Similar results appear in mass cultures derived from isolation cultures at various intervals after endomixis. When these mass cultures are subjected to standard conditions favorable to the occurrence of endomixis, the percentage of individuals which goes into endomixis is low soon after an endomixis, becomes high later, then falls, and finally reaches zero. Likewise, in order to induce endomixis in a given percentage of the individuals of a mass culture, the intensity of the inducing treatment must be great and prolonged soon after an endomixis; later, brief and moderate treatment will suffice; thereafter, the treatment must again be intensified and prolonged; and finally no treatment is successful. These three lines of evidence agree in demonstrating the same sequence of changes in the tendency to undergo endomixis as time since the last endomixis increases.

104. THE RELATION BETWEEN LOCOMOTION AND TEMPERATURE IN THE MARINE AMOEBA, FLABELLULA MIRA SCHAEFFER, WITH SPECIAL REFERENCE TO ADAPTATION TO TEMPERATURE. D. L. Hopkins, Duke University. (Lantern; 15 min.)

Once this amoeba has become adjusted to a specific temperature the rate of locomotion is constant. After adjustment to a specific temperature a sudden change in temperature of 7°C. or more causes the locomotion to pass through a series of fluctuations in rate, which continue for several hours before the amoebae become adjusted to the new temperature and the rate becoming constant again. The number and frequency of these fluctuations following sudden changes in temperature are greater the greater the temperature increase. After the amoebae have been subjected to each of the different temperatures for 10 hours the rate of locomotion is zero at 9°C. increases gradually to an optimum at 37.5°C., and then decreases to zero again at 43°C. If the rate of locomotion is observed before the amoebae have adjusted completely to the temperature, the rate-temperature curve will be very irregular, showing a series of optima and minima, but if observed after adaptation, a regular curve with but one optimum will be obtained. The fluctuations in rate after a temperature change are thought to be evidence that the adjustment to temperature involves a series of processes which proceed to equilibrium with the new temperature at different rates.

105. CHAOS CHAOS LIN., THE RARE GIANT AMOEBA FIRST SEEN BY ROESEL IN 1755. A. A. Schaeffer, Temple University. (Lantern and moving pictures; 15 min.)

This ameba was found in a New Jersey marsh in September, 1936. This is the fifth time it has been recorded since 1755 (H. V. Wilson, North Carolina, 1900; Penard, Switzerland, 1902; Kepner, Virginia, 1916; Schaeffer, Tennessee, 1916, and New Jersey, 1936).

A small culture of this ameba has been kept growing, and a few live specimens will be exhibited if the cultures can be maintained. Chaos chaos Lin. is about fifty times the volume of Chaos diffluens Mueller, the ameba commonly used in class rooms.

C. chaos is very similar to diffluens except in size and number of nuclei, and in the inconspicuousness of the contractile vacuoles in chaos. The nuclei in chaos vary from about fifty discoid nuclei of 35 μ long diameter to several thousand spherical nuclei of 10 μ diameter, depending upon size of the ameba, amount of parasitism, and other, undetermined factors.

The observed characteristics of chaos correspond so strikingly with the descriptions and drawings of Roesel's (size, ecology, mode of division when parasitized, movement, parasitism) that no reasonable doubt can be entertained any longer that this is the ameba Roesel saw. And the similarity of chaos and diffluens is so marked that there can be no escape from placing them in the same genus, if indeed they do not turn out to be one and the same species, chaos being a multinucleate stage of diffluens.

A motion picture film will show some of the characteristics of chaos.

106. STUDIES ON THE TRICHODINA (PROTOZOA, CILIATA) FROM THE DIGESTIVE TRACT OF THE SEA-CUCUMBER, THYONE BRIAREUS LESUEUR. Laura N. Hunter, University of Pennsylvania. (Introduced by D. H. Wenrich.) (Lantern; 15 min.)

Thyone harbors in its digestive tract many specimens of Trichodina (sp?) closely resembling Urceolaria synaptae Cuenot from various European species of Synapta. In Thyone two forms are distinguishable as follows, Type I: inhabits anterior secretory part of gut; bag-shaped; pellicle annulated; active locomotion through environment; frequent contraction; average length of 100 individuals, 112 μ, width, 75 μ; denticulate corona of large circumference; vegetative macronucleus elongate and somewhat E-shaped; type II: inhabits posterior absorptive part of gut; elongate, flattened; no definite pellicular annulations and few pellicular wrinklings; locomotion less active; contraction infrequent; average length of 100 individuals, 78 μ, width, 50 μ; denticulate corona of smaller circumference; vegetative macronucleus compact, bean-shaped.

Both forms undergo binary fission with mitotic division of the micronuclei. Chromosomes and spindle fibers are visible in stained preparations. In both forms pairs composed of one large and one small individual are found in association. Within each associate the macronucleus fragments and disintegrates and the micronucleus divides mitotically. In the large member of a pair, reorganization follows, during which process the micronucleus divides mitotically several times, giving rise to about eight macronuclear anlagen in addition to new micronuclei. Subsequent cell divisions appear to distribute these nuclei so that the final results of reorganization are new cells each containing one macronucleus and one micronucleus. The early macronuclear anlagen contain chromatic granules which are not noted in later anlagen.

Parasitology and Miscellaneous

107. ADOLESCARIAE FROM AGRIOLIMAX AGRESTIS. Bruce D. Reynolds, University of Virginia. (Lantern; 12 min.)

I. Three adolescariae of Brachylaemus virginiana were obtained from Agriolimax agrestis December 8, 1934. Polygyra thyroides has been found naturally infected with the larval stages of this trematode; also experimental infections have shown it to be a suitable intermediate host; but, this is the first time B. virginiana has been reported from the land slug.

II. All phases of the larval stage—sporocysts, cercariae and adolescariae—of Panopistus pricei have been found in Agriolimax agrestis, and is here reported for the first time. Heretofore Gastrodonta suppressa has been considered to be the intermediate host for this trematode, but since only adolescariae were found in that snail it seems more probable that A. agrestis plays this role.

108. SEX ORGANS AND REPRODUCTION IN CAMPELOMA RUFUM, A FRESH WATER SNAIL. Norman T. Mattox, University of Illinois. (Introduced by H. J. Van Cleave.) (10 min.)

Collections of Campeloma rufum were made at all seasons of the year from the Salt Fork River at Homer Park, Illinois, for gross anatomical and histological studies. No males nor male reproductive cells were found, all individuals showing definite female structures. The gonad, found on the columellar surface of the liver, shows definite ovarian cells. A U-shaped tube from the albumen gland to the uterus receives the oviduct near its proximal end. This duct leads into a glandular pocket, the shell gland, which in turn empties into the sac-like uterus. The uterus terminates near the edge of the mantle in a muscular vaginal tube. The previously undescribed ureter opens between the vagina and the anus. Intrauterine young were observed during all seasons of the year varying progressively from small veliger to fully shelled young. There seems to be a birth period during early summer, very few barren uterii were found. In view of the present evidence it is believed that this snail reproduces parthenogenetically. Specimens of Campeloma rufum from the Sangamon River in Illinois, Licking River in Kentucky and the Fox River in Wisconsin, all show the same absence of males. Other species of Campeloma and Viviparus having separate sexes were examined as a check on the technique and methods.

109. A NEW ORDER OF DIBRANCHIATE CEPHALOPODS. Grace E. Pickford, Yale University. (Lantern; 15 min.)

The status of the Vampyromorpha, a bathypelagic family of dibranchiate cephalopods, must be revised in the light of investigations of new material (Bingham Oceanographic Collection). None of the supposedly octopodan features previously described can now be sustained; among newly discovered characters the independent origin of the genital artery is more probably ancestral than octopodan. The theory that the retractile filaments represent the modified second arms is confirmed; these are not homologous with the tentacles, modified fourth arms, of Decapoda. The union of head and mantle is primary, not secondary as in the Octopoda; muscle fibers unite the nuchal cartilage with the shell which is internal and covers the whole dorsal surface of the visceral mass. The gills are decapodan; since the body surface has a thin pavement epithelium the small number of gill lamellae may be correlated with supplementary cutaneous respiration. Median ventral and lateral mantle adductor muscles, characteristic of Octopoda, are wanting; the muscle fibers are striated. From the spacious unconstricted coelom a slender canal, representing the siphuncular canal of Spirula, leads to a small sac in the apical region of the body. The open anterior chamber of the eye resembles that of oegopsid decapods; the absence of a white body is peculiar. The central nervous system shows a lack of condensation comparable to or greater than that found in Decapoda.

The Vampyromorpha can no longer be regarded as a suborder of the Octopoda but must be rated an equivalent order more nearly allied to the Decapoda.

110. A STUDY OF RELATIVE GROWTH IN NOTONECTA UNDULATA. Leonard B. Clark and A. H. Hersh, Union College and Western Reserve University. (Lantern; 15 min.)

Measurements on thirty-seven females of Notonecta taken at each of the six instars supply data for the study of relative growth in the single individual as well as in the average. The data conform approximately to the relative growth function, $y = bx^a$, in which x is the body length and y is in turn the body width, head width, length of first, second and third legs. The value of a for the average measurements is 1.074, 1.069 and 0.948 for the first, second and third legs, respectively. The value of a for the separate individuals varies from 0.981 to 1.168 for the first leg, from 0.947 to 1.217 for the second, and from 0.882 to 1.108 for the third leg. For these three sets of values as a increases b decreases. Log b plotted against a gives in each case a flattened sigmoid curve, which in its middle region approximates a straight line of negative slope. When y is taken as the femur length and x the length of tibia + tarsus of the same leg, the data for the three legs likewise conform approximately to the relative growth formula, and again log b is the same sort of decreasing sigmoid function of a. The final length of leg is a function of the growth equilibrium between the component parts. When a is taken as the independent variable it is found, for example, that a short final size of the metathoracic leg tends to be associated with the larger values of a, and vice versa.

111. SCIENCE IN FISH CULTURE. I. WITH SPECIAL REFERENCE TO ITS NEEDS. H. P. K. Agersborg, National Park Service. (15 min.)

Successful artificial propagation of fish involves: The building of economical and practical plants in which it is possible to eliminate as nearly as possible all physico-chemical and biological enemies common to fish in nature; the rearing units must, therefore, not be so large that direct observation of the fish in all stages of development is impossible; the food of the fish must be a balanced diet, varing with the age of the fish, and comparable to the normal diet of the species; the raised fish must be distributed economically and to waters consistent with the natural habits and needs of fish; the fish culturist, although not a trained scientist, must have learned how to observe accurately scientific facts, because his method of observation influences profoundly his interpretations and his attitude toward the whole problem of fish culture. If the fish culturist sees nothing or incorrectly, he has nothing to interpret or he interprets incorrectly. Proper management depends on proper observation and interpretation which will avoid the following common mistakes in fish culture: Overcrowding, improper food, improper feeding, unnatural shadeless or too large rearing quarters, indifference to disease producing factors, keeping of fish of different ages and sizes and kind in the same pond, expensive methods of planting of fish, and stocking of fish in situations unsuitable to the species regardless of size. The modern fish culturist should be familiar with biological principles and know how to apply them; he should be appreciative of and able to use profitably biological literature and know how to find it. Biologists who advise on fish culture should spend enough time with the practical worker so that he can base his advice on data observed by himself.

DEMONSTRATION PAPERS

Embryology

112. INTERGENERIC GRAFT HYBRIDS OF ECTEINASCIDIA AND PEROPHORA (ASCIDIANS). H. H. Plough, Amherst College.

At the Tortugas Laboratory of the Carnegie Institution last summer it was found that finely divided portions of the growing stolons of Ecteinascidia conklini, E. sp. and Perophora annectens placed in sea water in glass containers would join into an interconnected mass and eventually regenerate normal colonies. Equal portions of these stolons for each of two of these genera in all three possible combinations were placed together. Successful composite colonies were developed in every combination. Zooids developing on the composite regenerated strands usually resembled one or the other type, but in two cases the zooids showed the intermediate character of chimaeras. In the short time available no zooids developed to sexual maturity. Several mounted specimens will be shown.

113. REGENERATION AND DIFFERENTIATION IN CRUSTACEA. Hugh H. Darby, College of Physicians and Surgeons, Columbia University.

See abstract no. 67.

114. INCOMPATIBILITY IN SPECIES AND LOCAL RACES OF AMBLYSTOMA DURING PARABIOSIS OR FOLLOWING TRANSPLANTATION OF TISSUES. R. R. Humphrey and R. K. Burns, Jr., University of Buffalo School of Medicine and University of Rochester School of Medicine and Dentistry.

Larvae of Amblystoma tigrinum and A. punctatum of certain geographical races, united in parabiosis, develop harmoniously until onset of metamorphosis, when a violent incompatibility, affecting the punctatum member, arises. The same is true of A. punctatum larvae, bearing large dorso-lateral grafts of body wall from A. tigrinum of the upper Mississippi valley region. In either case, when A. tigrinum of the Tennessee strain are used, no reaction occurs.

The reaction is characterized by edema, desquamation of epidermis, and circulatory disturbances resulting in necrosis of digits or entire limbs, widespread breakdown of peripheral vessels occurs, and death follows almost without exception.

However, in parabiotic pairs death is prevented by prolonged immersion in mercurochrome solutions, which apparently aid by limiting infection. Healing then occurs and missing parts regenerate. Once overcome, the reaction does not recur.

The reaction can be duplicated in normal premetamorphic punctatum larvae by introducing into the peritoneal cavity skin from a tigrinum of the region mentioned, or skin from a transplant in a host exhibiting the reaction. This with other evidence indicates the reaction to be due, in hosts bearing grafts, to secretions from the cutaneous glands of the transplant. In parabiotic pairs it is attributed to the same agents reaching the punctatum member through the common circulation.

The demonstration includes charts, protocols, photographs, specimens and microscopic preparations.

115. THE GROWTH OF AN URODELE LARVA AS ILLUSTRATED BY TRANSFORMED COORDINATES. Oscar W. Richards and G. A. Riley, Yale University.

A demonstration of D'Arcy Thompson's method of transformation of coordinates to show the changes during larval growth of Amblystoma punctatum using the data of Dr. R. F. Blount (J. Exp. Zoöl., 1935, vol. 70, pp. 131–182). The changes in the coordinates illustrate the activity of growth and permit comparisons of form changes with time, in contrast to the comparisons of related adult forms by Thompson.

116. MICROSCOPICAL PREPARATIONS OF BONE MARROW OF TURKEY, SHOWING THE RELATION OF LYMPHOID NODULES TO BLOOD PRODUCTION. H. E. Jordan, University of Virginia.

Numerous nodules occur in the marrow of the tibia of the young individual. These nodules are related both to granulocyte and erythrocyte production. The nodules comprise collections of larger lymphocytes enveloped by a mantle of smaller lymphocytes. Only the larger lymphocytes proliferate. The smaller daughter lymphocytes migrate into the intranodular capillaries and are carried to the extranodular sinuses where they grow and differentiate into erythrocytes. Migrants to the extranodular stroma differentiate into granulocytes. The thrombocytes develop from small lymphocytes in the venous sinuses.

117. DEVELOPMENT OF THE TRANSPLANTED INNER EAR RUDIMENT OF THE RABBIT EMBRYO. A. J. Waterman, Williams College.

The development of the transplanted inner ear rudiment of young rabbit embryos has been followed from the eighth to the eleventh day of gestation by means of omental and kidney grafts. Young and mature rabbits were used as hosts and the grafts were recovered after 8 to 15 days of growth.

The transplants grow and differentiate in the abnormal environment and the membranous epithelium becomes surrounded by cartilage. Morphogenesis and histogenesis do not keep pace with each other; the former showing more evidences of retardation. Environmental factors influence the expression of the graft potentialities. The shapes of the graft ears of any one stage are highly variable while the grafts of the older rudiments are more typical in appearance. This is correlated with the advanced organization of the older rudiments and their resistance to the environment. These vary from a sac-like structure with an endolymphatic duct to a recognizable labyrinth with two semi-circular canals, endolymphatic duct and sac, utriculus, sacculus and rudiment of the cochlea. The crus commune and generally the horizontal canal are lacking and the ampullae are poorly outlined. The graft labyrinths of a particular stage vary widely in size. Histologically the more complete ears show sensory areas, with or without nerve innervation, cupulae and typical epithelium. The host reaction is that of a typical foreign body type.

Cardboard-paraffin models of certain of the more typical labyrinths and optical line remonstructions of others will be demonstrated together with photographs showing something of the histological development.

118. A CYCLOPS LAMB. A. M. Reese, West Virginia University.

A Hampshire lamb was received in too advanced decomposition to permit the study of more than the skull and the gross features of the eye.

No nasal openings and no proboscis were present. The eye was single, with one optic nerve. The eye muscles, so far as could be determined, were a curious mixture and impossible to name.

Owing to the almost complete absence of the bones of the upper jaw the skull, with the exception of the mandible, was curiously like the human skull. The frontal bone was unpaired, while the alisphenoid and interparietal were greatly enlarged.

Physiology

119. STIMULATION OF THE HYPOPHYSIS AND GENITAL GLANDS IN THE DUCK BY ELECTRIC LIGHT. EFFECT OF THYROIDECTOMY ON THE TESTIS AND LIVER. Jacques Benoit, Yale University School of Medicine. (Introduced by Edgar Allen.)

The illumination with common electric light bulbs (15 to 18 hours per day) induces a marked increase in the size of the testis in immature male ducks. The immature testis may show complete spermatogenesis after 2 weeks and a volume increase of sixty to eighty times. Meanwhile the Leydig cells and sex organs (epididymis, vas deferens, penis) grow considerably.

Full maturation of the ovaries and egg laying may be induced in 4 to 5 weeks by the same treatment in immature females.

Prehypophysectomy completely stops the stimulating action of light upon the gonads. Also the implantation of the prehypophysis into immature female mice demonstrates its activation by the light treatment.

The light acts through the ocular region, but its stimulating action is maintained after cutting both optic nerves or removing both eyes.

The direct and localized illumination of the pituitary area through a thin glass tube fixed in the orbit stimulates the activity of this gland and testicular growth.

Among the visible light rays the longer red and yellow rays were proven active.

After thyroidectomy, the testis growth induced by light is greatly reduced.

In illuminated or non-illuminated thyroidectomized ducks, the weight of the liver increases considerably. An increase of two or three times may be obtained in 3 or 4 weeks by the increase of its components, especially lipids.

120. PHENOMENA OF REGENERATION AND GROWTH, INJURY AND RECOVERY, CONTRACTION AND RETRACTION OF STRIATED MUSCLE FIBERS.

Carl C. Speidel, University of Virginia Medical School. Motion picture films.

No abstract received.

Endocrinology

121. THE EFFECT OF PITUITARY HEBIN UPON THE REPRODUCTIVE SYSTEM OF THE CHICK EMBRYO.

L. V. Domm and E. A. Dennis, The University of Chicago and American University.

Injections of pituitary hebin were made into albumen and allantoic cavity of incubating eggs. They were made at varying ages, daily and alternate days, beginning at 48 hours and majority terminating on ninth day. Number of injections varied from one to six, volume from 0.05 to 0.3 cc. and dosage from 5 to 30 R.U. Most of embryos were killed and examined on eighteenth day the rest on the nineteenth. Brown Leghorns and a hybrid variety with sex linked plumage were employed.

Gross examination revealed hypertrophy of gonads particularly pronounced in cases receiving higher concentrations. The degree of hypertrophy varied and was more general and pronounced in females than males. Particularly significant was the striking response of the rudimentary right gonad not previously encountered in our postembryonic injections. This gonad usually small was frequently larger than the normal left.

Histologically, experimental ovaries show a narrow, irregular, frequently discontinuous, layer of cortex clearly distinguishable from underlying medulla. Medullary hypertrophy is conspicuous and greater size of experimental ovaries is primarily due to its growth. The right gonad most frequently consists entirely of medullary tissue which varies from a rather loose arrangement with numerous large irregular spaces to a firm condition with few small spaces. In a number of instances small surface areas of cortex were encountered.

Histologically, hypertrophied testes show no significant modification of gametogenetic tissue though they do reveal pronounced hypertrophy of interstitial elements.

Accessory sex characters in general reveal no response. Hybrid gonads of both sexes were more responsive than leghorn.

122. FEATHER SEQUENCES GROWN FROM SINGLE FOLLICLES IN THE SADDLE OF THE HYBRID CAPON AND FEMALE HORMONE MODIFICATIONS.

Mary Juhn, The University of Chicago.

Series of feathers grown from single follicles in the saddle of a hybrid capon (F_1, Barred Rock ♂ — Brown Leghorn ♀), will show a) the manner in which barred succeeds exceptional and vice versa, and b) the absence of pattern correspondence in successive exceptional feathers.

Photographs of mounted hybrid saddle feathers with regions of simultaneous formation in the germ projected on the feather vane demonstrate the simultaneous presence of barred and leghorn components in the formative zone, the collar, of the feather germ.

A number of saddle feathers regenerating in the presence of female hormone illustrate the variation in response obtained when a) the injections of the hormone are commenced before regeneration sets in, and b) when the injections are started after regeneration of the male feather, barred and exceptional, is well under way.

123. FURTHER EXPERIMENTS ON HORMONAL CONTROL OF THE SEASONAL PLUMAGE CHARACTERS IN PYROMELANA FRANCISCANA (AFRICAN WEAVER FINCH). Emil Witschi, State University of Iowa.

The males of Pyromelana franciscana wear a gaudy cock plumage during the breeding season and an inconspicuous hen plumage during the season of sexual quiescence. Castrate males and females show the same rhythmical change, though the molting processes are more irregular. Normal females retain the henny plumage throughout the year. Feathers of males, females and castrates will be exhibited to show 1) that during the quiescent season feathers will grow in the cock type as long as the bird is injected with gonadotropic hormone, and 2) that during the breeding season daily injections of theelin, theelol or dihydrotheelin cause feathers to assume the henny characters. One may conclude from these experiments, that the hen plumage represents the neutral type. A high level of gonadotropic hormone changes it into the cock type, though the simultaneous presence of adequate amounts of female sex hormones prevents this change.

124. THE HISTOLOGY OF PRECOCIOUSLY ACTIVATED THYROIDS OF THE TADPOLE. William Etkin, City College and American Museum of Natural History.

See abstract no. 90.

125. SPECIFICITY OF THE PITUITARY GONADOTROPIC FACTOR IN AMPHIBIANS AS DEMONSTRATED BY IMPLANTS OBTAINED FROM FISHES. Charles W. Creaser and Aubrey Gorbman, Wayne University.

An investigation is being made of the response, by extra-seasonal ovulation, of the frog Rana pipiens to pituitary substances from various sources. Frogs capable of ovulation failed to ovulate when treated with the following preparations: fresh pituitaries from Stizostedion vitreum, Perca flavescens and Cristovomer mamaycush; and immediate transplants from live Perca flavescens. Experiments are in progress with the fish Lepisosteus osseus inasmuch as extra-seasonal ovulation of R. pipiens by fishes of this genus is indicated in uncontrolled experiments of Wills, Riley and Stubbs. This data with other not reported here indicates a remarkable difference in ovulation response of R. pipiens to pituitary substance from different sources.

126. VAGINAL CHANGES, HEAT AND THE TIME OF OVULATION IN THE GUINEA PIG. William C. Young and Edward W. Dempsey, Brown University.

Vaginal smears made at known times with respect to heat and ovulation have been studied.

Subdivision of the old stage I seemed desirable; Ia composed of superficial cells only; Ib, early, when less than 25% of the cells are cornified; Ib, middle, when 25% to 75% are cornified; Ib, late, when more than 75% are cornified.

Heat prior to Ia or during stage III is rare. It occurs most commonly during stages Ia to Ib, middle, inclusive, but the association is not sufficiently definite to make the method satisfactory for positive identification of heat. This depends upon examination for the copulatory response.

When smears were made at ½-hour intervals beginning immediately after rupture of the membrane heat frequently was later with respect to the vaginal condition than in undisturbed animals. This is attributed to the advancement of vaginal changes rather than to the delay of heat.

Ovulation is associated with heat rather than a definite vaginal condition. Consequently, the latter does not indicate accurately the time of ovulation.

The suggestion that within fairly broad limits vaginal changes and heat are separable is confirmed, and is reconcilable with recent experimental work on the cause of heat.

When heat occurs early with respect to vaginal changes its average duration is significantly longer than when it occurs later. This suggests that following an early heat-stimulus the oestrin-conditioning may be optimal. Conversely, when the stimulus occurs later, conditioning may no longer be optimal and heat tends to be short.

Cytology

127. CYTOLOGY OF ABNORMAL GROWTHS PRODUCED BY X-RAY IRRADIATION OF GRASSHOPPER EGGS. E. Eleanor Carothers, University of Iowa.

Eggs of Melanoplus differentialis were given either 250 r. or 300 r. in a single exposure to x-rays at ages varying from 5 to 9 days. The eggs were then allowed to continue development at 25°C. (T. C. Evans has shown that about 50% of such eggs produce embryos which are abnormal morphologically). Samples were fixed at 24-hour intervals. Twenty-four hours after irradiation numerous cells with pycnotic nuclei are scattered throughout the germ band. Such cells are either engulfed by other embryonic cells, absorbed in situ or cast out of embryo. Within 48 hours certain cells show liquefaction of cytoplasm and within 5 days lesions due to cytolosis appear. This cytolosis may be caused by: 1) injury to cytoplasm by x-rays, 2) to injury or destruction of other cells upon which the cytolized cells are dependent, 3) to a toxic effect from cells with pycnotic nuclei which have been phagocytized, or 4) to a general toxic effect of degenerating tissue. Cells with pycnotic nuclei have practically disappeared within 5 days but at later stages they again become abundant; now, however, they occur in groups which like the above-mentioned cytololytic lesions may result in isolation of groups of healthy cells from their normal associations in the embryo. These groups of normal but misplaced cells appear to give rise to structural abnormalities. The original pycnosis is due to the stage of mitosis at the time of irradiation.

128. Isolation of interkinetic chromosome pairs from germinal vesicles of the ovarian eggs of Rana pipiens. William R. Duryee, Columbia University.

Elastic chromosome pairs, of higher specific gravity than the rest of the nucleus, may easily be isolated by hand under a dissecting microscope at any stage during the year or more they remain in the ovary. This fact is offered as evidence of their intermitotic continuity. Fresh, unfixed chromosomes, some isolated on a slide and others in situ in isolated nuclei, will be shown. Complete chromosome pictures may thus be had from the living animal in less than 20 minutes. These techniques should also prove useful to geneticists as well a to colloidal chemists.

129. The non-homogeneous character of the nuclear membrane. William R. Duryee, Columbia University.

Isolated fragments of the nuclear membrane in the ovarian eggs of Amphibia and Teleosts form remarkable sac-like protuberances reversibly with pH changes. These sacs are arranged mosaically over the surface of the sphere and stain differentially with crystal violet from the rest of the membrane. Their appearance explains the erroneous conclusions of some authors that the nucleus may be capable of amoeboid movement.

130. New criteria for distinguishing living and dead cells. B. J. Luyet and P. M. Gehenio, St. Louis University.

Presentation of 1) Ultraviolet photographs of naturally pigmented, unstained, living and dead cells; and of non-pigmented, vitally stained, living and dead cells. 2) Color photographs of cells stained by a differential staining procedure developed to give violent contrast between living and dead cells.

131. Cytology and development of flame cells in the redial generation of a trematode. Charles H. Willey, New York University.

Cercaria poconensis develops in rediae and the adult stage is unknown. In connection with studies on the intimate structure of flame cells from sections of these rediae, an attempt has been made to find cytological evidence in support of the current hypothesis that in the development of the excretory system, these cells divide by mitosis, followed by a splitting of the collecting tubule. The rediae in various stages of development possess from two to twelve flame cells, and if the increase in number is by division of previously differentiated flame cells, ten such divisions must have occurred in the development of each redia. More than a thousand flame cells from several hundred immature rediae have been studied. No evidence of cell division was found and the nuclei gave no indication of mitosis. Differences in cell size and nuclear size of flame cells found near each other within the same redia, and the presence of small, isolated flame cells apparently in an early stage of differentiation suggest their origin from undifferentiated cells in the parenchyma. Examination of sections of all stages in the development of rediae affords no evidence in support of the belief that flame cells arise by division of previously differentiated flame cells.

132. A STUDY OF THE BACTERIAL AND ALLEGED MITOCHONDRIAL CONTENT OF THE CELLS OF THE CLOVER NODULE. E. DeWitt Miller, University of Virginia.

See abstract no. 87.

133. CUPULAE ON THE LATERAL-LINE ORGANS OF FUNDULUS HETEROCLITUS. Martha Denny, Connecticut College. (Introduced by Pauline H. Dederer.)

Cupulae on lateral-line organs are easily observed in Fundulus because of the superficial position of the organs. Cupulae have been found on living as well as fixed specimens and observations made on the possible origin of the cupular material.

This structure is similar to the cupula of the crista ampullaris of the vertebrate ear and consists of a cone of gelatinous material attached to the summit of the sense organ. The length of the cupula and its elasticity vary in different specimens. In all cases longitudinal striations are observed in the jelly. Evidence is presented indicating that the cupula does not consist of mucus, of a secretion from the sense cells, or of fused sensory hairs. Its origin from supporting cells and intercellular substance is considered probable.

134. THE EFFECT OF CHLORETONE ON TISSUE PRECEDING FIXATION. Robert T. Hance, University of Pittsburgh.

That the normal irritability of protoplasm might result in artefacts when living tissue was fixed in the usual ways led to experiments on anaethetization with chloretone preceding fixation. The slides on exhibit show the experimental tissues to be somewhat less shrunken than the controls.

135. AN IMPROVED AND INEXPENSIVE MICROTOME KNIFE. Robert T. Hance, University of Pittsburgh.

Joiner (rotary plane) blades have been found to be of superior quality steel and excellent for sectioning. A 6-inch blade may be cut in half making the cost per unit less than 50 cents. A satisfactory holder for these blades is a 5-inch length of 'piano' hinge which costs about 25 cents. The blades are readily sharpened on hones usually obtainable at 10-cent stores. These entire outfits cost less than one dollar and make it possible for every student to have his own sectioning equipment.

136. HOW THINGS GROW. W. J. Baumgartner, University of Kansas. (Shown in room 11 at 4.00 P.M.)

A moving picture film showing the division of living cells of the grasshopper testis. The details of structure and the movement of the chromosomes from the metaphase position to the poles and the reconstruction of the nucleus are shown in detail. The cleaving of the metachondrial threads stands out plainly. Individual chromosomes can be followed throughout the metaphase and anaphases. The film has a very definite value as a means of teaching cell division.

Protozoölogy

137. Cultures of living Chaos chaos Lin. A. A. Schaeffer, Temple University.

The culturing of this ameba was attended with considerable difficulty and it still requires constant attention to keep it from dying out. There are, however, good prospects of working out suitable cultural conditions so that it may be grown in mass culture in sufficient numbers for research purposes and perhaps for class use.

Roesel's original description and colored drawings will also be exhibited.

For purposes of comparison, a culture of Chaos diffluens will be available for examination.

Miscellaneous

138. Studies on Clinostomum. V. The cyst of the yellow grub of fish (C. marginatum). George W. Hunter, 3rd and H. Clark Dalton, Wesleyan University.

Two general types of cyst walls are encountered in studying larval helminths. The first type is made of two layers, an inner hyaline sheath presumably elaborated by the parasite, which in turn is surrounded by an outer one of connective tissue. The second consists solely of connective tissue laid down by the host. The yellow grub of fish (Clinostomum marginatum) has cysts of the latter type.

A comparative study of cysts of the same age from four different species of fish show the following: 1) Cyst walls are composed of fibrous connective tissue with characteristic flattened nuclei, although the amount of connective tissue varies in the different hosts. 2) Cyst walls are thinnest in the small-mouthed bass (M. dolomieu), but more independent of muscle tissue in the guppy (Lebistes reticulatus). 3) The parasites grow more rapidly in comparable locations in the guppy and common sunfish (E. gibbosus) than in the bass and banded sunfish (E. obesus). 4) In the banded sunfish at least, the muscle bundles immediately surrounding the cyst are thinner and tend to become separated by the formation of connective tissue between them. 5) Comparable data on year-old cysts from the guppy, common and banded sunfish are included.

139. The larval form of Foleyella americana Walton, 1929. A. C. Walton, Knox College.

Rana sphenocephala specimens collected in Florida during the spring of 1936 yielded a considerable number of examples of Foleyella americana Walton, 1929. At the same time, examination of the blood of the same specimens showed an abundance of filariid larvae which possessed the characteristic structures of a form related to Foleyella Seurat, 1917. Feeding of eggs to Rana pipiens from Illinois that showed no parasites in their blood, gave rise to a blood picture of similar larvae after an interval of 2 weeks. Five weeks after the feeding of the eggs, adult examples of Foleyella americana were obtained from these specimens of Rana pipiens. Rana pipiens from Illinois has not been found to harbor specimens of Foleyella spp. as a natural infection, thus indicating that the larvae found are undoubtedly those of Foleyella americana Walton, 1929.

PAPERS READ BY TITLE

Embryology

140. THE ROLE OF l-ASPARTIC ACID IN THE DEVELOPMENTAL GROWTH OF OBELIA GENICULATA. F. S. Hammett and Hans Schlumberger, Marine Experimental Station of the Lankenau Hospital Research Institute, North Truro, Massachusetts.

Fifty-eight experiments with close to 30,000 Obelia hydranths half of which were exposed to l-aspartic acid in six different concentrations show that the amino acid is a specific participant in some process concerned with differentiation expression; that it has no apparent effect on any other developmental activity; and that the metabolic activity of maintenance is essentially unaffected thereby. Its influence on development is similar to that of its next higher homologue d-glutamic acid. Suggestive is the fact that the removal of a CH_2 group is accompanied here with reduction on stimulation effect on regression of the complete feeding hydranths as it was in the earlier experiments with glycine and d-alanine. Thus in the compounds with one less CH_2 group such as glycine and l-aspartic acid regression in the test animals was increased over that in the controls to much less degree than it was in the case of d-alanine and d-glutamic acid of one more CH_2 group in the carbon chain.

141. THE SURFACE LAYER OF THE GERMINAL VESICLE OF THE EGG OF SABELLARIA VOLGARIS. A. J. Waterman, Williams College.

In distilled water osmotic equilibrium of the germinal vesicle is reached immediately on swelling and cytolysis of the egg. Very little if any further swelling occurs in the free vesicle. The surface layer, which otherwise disappears during maturation, hardens and may persist unchanged for hours in distilled water, sea water or glycerine. It has marked thickness even when swollen as compared with the surface layer of an exovate. The layer does not dissolve at once in distilled water and glycerine as does the vitelline membrane of the newly shed egg. Within the intact egg, the plastic vesicle is irregular in outline and its shape changes as the egg rounds up and maturation is initiated.

If returned to sea water within a very few minutes of exposure to distilled water, the vesicle will sometimes shrink to about its original size. In hypertonic sea water further shrinkage may occur and the surface becomes more or less puckered. These changes can be produced only within a few minutes after liberation of the vesicle. If the series of changes is made quickly enough, the puckered vesicle will occasionally swell again in distilled water but much more slowly than when first removed. Ordinarily the puckering does not last long. Occasionally the surface layer ruptures and one or infrequently two exovates form. When this happens, the vesicle shrinks somewhat. The surface layer of the vesicle thus seems to exhibit some of the characteristics described for the egg surface and membranes. The free vesicle is probably non-living.

142. PROTANDRY IN THE VIRGINIA OYSTER. W. R. Coe, Yale University.

Samples of young individuals of Ostrea virginica of known ages from Cape Cod to the Gulf of Mexico show that the species is mainly but not exclusively protandric, the proportion of males at the first breeding season ranging from 70 to 97% under different environmental conditions. The age at first sexual maturity varies from 8 to 48 or more weeks. At Long Island Sound a small proportion of young oysters, which in that area usually breed at the age of 1 year, may experience two functional sexual phases in the same season, first functioning as males and immediately thereafter as females. A few others are functionally hermaphroditic and capable of self-fertilization.

Histological examination shows that in those individuals which first function as females, the activities of the spermatogenic cells of the primary bisexual gonad are inhibited, with a correlated proliferation of the primary ovogonia. Partly due to the change in sexual phase and partly to the later maturity of many of those individuals first functioning as females the ratio of males decreases during the breeding season at each locality. The greater the length of the first growing season and the more favorable the environmental conditions, the larger is the proportion of females the following year, since the primary male phase is more promptly initiated and either aborted or completed under such conditions.

The sequence of functional sexual phases in this oviparous species is evidently more closely similar to that of the viviparous oysters than has been previously reported.

143. SUSCEPTIBILITY OF BRUCHID (COLEOPTERA) EGGS TO KCN. Alfred Brauer, University of Kentucky.

Eggs of the pea weevil Bruchus quadrimaculatus incubated at 28°C., are most susceptible to KCN (1/100 mol.) during the first 6½ hours incubation. During this interval susceptibility drops slightly. Development is completely arrested in 85% of the cases after 25 minutes treatment. From 7½ to 12 hours incubation the eggs are highly resistant as regards complete arrest. One and one-quarter hours treatment is required to halt development in 90% of the eggs. After shorter treatment these eggs are highly anomalous in antero-posterior axial development of embryo. The period from 15 to 18 hours incubation is one of higher susceptibility than the former period. Sixty minutes treatment completely arrests development of over 80% of the eggs. Bifida of the ventral plate is the principal anomaly resulting from sub-lethal dosage. After this period eggs are highly resistant to treatment, though anomalies of mouth parts result after treatment at 24 hours incubation.

Each of the enumerated periods corresponds to a phase of development as follows: the first period to blastokinesis and to embryonic determination; the second to blastoderm formation and to first axial development; the third to middle germ layer formation and to ventral nerve cord development; the fourth to growth of mouth parts.

144. REPRODUCTIVE CYCLES AND SUPERFOETATION IN THE POECILIIDAE, A FAMILY OF TROPICAL AND SUB-TROPICAL OVO-VIVIPAROUS FISHES. C. L. Turner, Northwestern University.

The Poeciliid fishes maintained in aquaria produce broods of young at regular intervals varying in different genera from 50 days to 5 days. The actual time required for development of ova and of embryo up to time for birth in different genera is about the same.

In species with the longest interval between broods, ovocytes destined to form the next brood are very minute at the time of birth of a group of embryos. More than 2 weeks of growth and differentiation are required before they are ready for fertilization. In species having intermediate intervals between broods (Lebistes reticulatis, etc.) the group of ovocytes destined to produce the next brood are larger and more differentiated and are ready for fertilization about 7 days after the voiding of the previous brood.

In species producing broods at 15-day intervals (Poecilistes pleurospilus and Poeciliopsis infans) the next younger group of ova are fully matured and are fertilized before the voiding of the previous brood so that two separate broods are present in the ovary at the same time (superfoetation). In the extreme case of Heterandria formosa there are six small broods separated from each other by recognizable stages of development. Broods of one to ten embryos are born every 4 to 8 days.

The Poeciliid fishes arose under tropical conditions and have not developed the annual seasonal reproductive cycles of fishes of the temperature zones but have retained instead the short often-repeated cycles.

145. REPLACEMENT OF THE ANTERIOR LIMB BY A GRAFTED MEDULLA IN THE EMBRYO OF THE ANURAN DISCOGLOSSUS, AND ITS RELATIONS WITH THE LAW OF BILATERAL SYMMETRY OF NEURONS. Raoul M. May and Alice Frank, University of Paris.

Neurogenesis of the medulla, which was grafted at the tail bud stage, occurred under conditions which were close to the normal ones, in situ; but it was the new environment in which the graft found itself which determined the paths followed by the nerves so formed.

The presence of the supernumerary medulla in the region of the right forelimb completely inhibited the formation of the latter at metamorphosis. On the operated side, the nerves which form the brachial plexus, as well as their ganglia, were very much reduced, and innervated muscles of the shoulder region.

Reduction of the brachial plexus was concomitant with a ponderable reduction of the spinal cord on the affected (right) side and level. The total number of cells was reduced (17%). The numerical reduction of the large motor cells was easily observed on the affected side and level. The ponderable asymmetry (9 to 21%), very evident at the level of the reduced brachial plexus, diminished in the posterior segments; symmetry of the cord was perfect in the segments where was normally present, on both sides, the lumbo-sacral plexus.

These results show that the forelimb, like other innervated peripheral organs, follows the law which May formulated in 1933: "In development, bilateral symmetry of the sensory and motor neurons depends closely on the bilateral symmetry of innervation of the peripheral organs."

146. MATURITY AND SECONDARY SEX CHARACTERS IN ADULT PARABIOTIC TWINS OF SALAMANDERS. Emil Witschi, State University of Iowa.

Ten pairs of twins of the combination Ambystoma tigrinum + A. jeffersonianum were raised to sexual maturity, and eight pairs in which an Axolotl was combined either with A. tigrinum or with A. maculatum were kept alive for from 1 to 4 years. This material answers some questions on hormonic interactions which were left open in the previous studies on pairs preserved while still immature. Gonad development in each twin follows its own specific tempo as long as both are in the juvenile period. When the faster one approaches the period of maturity the picture changes. The lagging co-twin speeds up to the extent that both reach maturity at the same time. Obviously maturation, but not prepuberal development is controlled by gonadotropic hormones.

Of particular interest are five pairs, all over 1 year old, each of which is composed of a mature tigrinum male and a jeffersonianum female. The ovaries of the latter are sterile, without germ cells, and extremely reduced. However, the vasa deferentia and cloacal glands, are those of mature males; which proves that male sex hormone is transmitted from the tigrinum to the jeffersonianum twin. This abundant transmission of gonadotropic and of sex hormones is in contrast with the conditions observed in parabiotic rats. The difference is referable to the relative quantities of blood that are exchanged. Amphibian twins, which are grafted together at an early embryonic stage, develop much more extensive vascularly connections than rats which are united 1 month post partum or even later.

147. DIFFERENTIATION IN VITRO OF THE ISOLATED MESENTODERMAL LAYER OF THE CHICK BLASTODERM. Dorothea Rudnick, University of Rochester.

The entoderm, with adherent mesoderm, was removed from chick blastoderms of primitive streak and early head process stages, and cultivated on plasma embryonic extract clots for 2 to 4 days. Twenty-eight examples have been studied. In ten of these cultures, the tissue merely formed an epithelial or epithelium and fibroblast membrane on the surface of the clot. In the other eighteen cases, one or more characteristic vesicles, in addition to a membrane adhering to the clot, developed. One or two pulsating heart masses usually are found within the vesicles of such a culture. After 4 days in vitro, vesicles tend to flatten out against the clot, heart masses to spread out as a central sheet in this membrane, where they soon cease pulsating and dedifferentiate.

Sections of these cultures show in most cases progressive differentiation of mesodermal elements only: heart muscle, blood cells, endothelial channels. The production of vesicles is evidently due to the balooning out of these tissues, which carry the covering entoderm over them as they enlarge. The non-yolky entoderm cells remain as flat as when explanted. However, in one case, unmistakable liver cords have developed, in connection with a heart of exceptionally normal form.

Incomplete studies of the behavior in vitro of fragments of blastoderms containing ectoderm show that columnar epithelial may form on the outside of vesicles; hence the failure of gut epithelium to differentiate in this situation is not to be attributed directly to the experimental environment.

148. THE GLYCOGENIC FUNCTION OF HEPATIC TISSUE IN NORMAL LIVERS AND IN CHORIO-ALLANTOIC GRAFTS. A. J. Dalton, Western Reserve University.

One hundred and fifty-four normal livers from chick embryos varying in age from 6 to 18 days incubation were studied for their glycogen content. Best's carmine stain was used after celloidin embedding. A series of chorio-allantoic grafts of hepatic tissue was studied in similar fashion.

In the normal series, glycogen was first identified in the hepatic cells of the 6-day embryo. Insulin, not identifiable in the blood stream before the eleventh day, would not appear to be responsible for the onset of glycogen synthesis and storage in the hepatic cell of the chick.

Livers of all embryos of 7 and 8 days incubation and of 14 to 18 days incubation contained glycogen in readily identifiable quantities. During the period from the ninth to the thirteenth day, considerable variation in glycogen content was found. Ninety per cent of the livers on the ninth day, 70% on the tenth, 65% on the eleventh, 31% on the twelfth, and 70% on the thirteenth day contained glycogen. The relatively low glycogen content of livers during this period appears to be correlated with the increase in metabolic rate of chick embryos which is known to occur during the period from the ninth to the twelfth day of incubation.

Glycogen was identified in all grafts with a total age of 8½ days or more, but could not be identified in any of the younger grafts. Normally developing hepatic tissue then, acquires the capacity to synthesize and store glycogen at an earlier age than hepatic tissue transplanted to the chorio-allantois.

149. DEVELOPMENT OF THE OVIDUCTS OF THE RED-TAILED HAWK, BUTEO BOREALIS BOREALIS (GMELIN). Allan J. Stanley, State University of Iowa. (Introduced by Emil Witschi.)

The development of the oviducts of the red-tailed hawk has been investigated. At 16 days incubation both oviducts are present and extend from points anterior to the ovary to the cloaca. At this stage they are of even size and length. A different growth rate is soon established on the two sides, the right oviduct developing little if any further in its long axis; the left oviduct, however, continues to grow but does not quite keep pace with the lengthening of the other parts of the urogenital system. At 20 days incubation the anterior end of the right oviduct lies at the level of the posterior end of the left ovary while that of the left oviduct lies even with the anterior end of the ovary.

At 24 days incubation the anterior end of the right oviduct lies just posterior to the mesonephros while at the 28-day stage it has been withdrawn to a point midway between the posterior end of the mesonephros and the cloacal attachment. At hatching only a rudiment remains attached to the cloaca.

After hatching, the left oviduct develops more slowly and, as the body lengthens, is also drawn backward until the ostium lies posterior and lateral to the ovary. As the body cavity increases in width the tubal ligament, being attached to the latero-dorsal body wall, changes its direction of support farther away from the midline. The relative backward movement of both oviducts is due to slower growth than that of the body cavity.

150. SPERMATOGENIC ACTIVITY IN FIRST YEAR (JUVENILE) SPARROWS, PASSER DOMESTICUS. Gardner M. Riley, State University of Iowa. (Introduced by E. Witschi.)

A mock breeding season in the fall or early winter is found to occur in juvenile male sparrows of the Iowa region. This is of interest since at the same time the testes of adult sparrows remain quiescent. Externally such precociousness becomes evident by the darkening of the bill pigmentation. The testes are larger than those of the quiescent adult. Adult testes, at this time, are between 1 and 1.5 mm. in length while those of juvenile birds range up to 10 mm. In 1935 (November 17th) as well as 1936 (October 2nd and 16th) testes of juvenile sparrows were preserved in the early morning hours. In every case, with one exception, actively dividing spermatogonia were present. This activity at a time quite remote from the normal breeding season is correlated with a high amount of daily sunshine which is characteristic for this part of the country at this time of the year. The change from a period of dull days to a period of clear days simulates the normal spring condition (or experimentally lengthened light periods) which has been shown to stimulate testicular development in birds that are in a responsive condition. The fact that in the fall most young sparrows are in a responsive condition, in contrast to males older than 1 year, had been borne out previously by one of our experimental series (Riley, '36). Later in the year, when the amount of daily sunshine again decreases, the stimulated testes revert to the quiescent state.

151. THE DIFFERENTIATION IN THE RAT OF GONAD PRIMORDIA GRAFTED TO GONADECTOMIZED HOSTS. Adrian Buyse, University of Rochester.

It has been shown previously that morphologically indifferent fetal rat gonad primordia when transplanted to a subcapsular position on the kidneys of sexually mature rats, differentiate according to their original zygotic determination irrespective of the sex of the host. To determine whether or not gonad differentiation of a similar order could be obtained in gonadectomized hosts, a parallel series was undertaken employing castrates of both sexes as hosts. Thirty-nine cases recovered from castrate males and fifty-three cases from spayed females are here reported. Twenty-one testes, twelve ovaries, one ovotestis, four in which sex cannot be identified and one with no gonad are represented in the castrate male series. Thus a ratio of twenty-one testes to eighteen ovaries of varying degrees of differentiation is obtained. Twenty-four testes, nine ovaries, one ovotestis, six grafts of undetermined sex and thirteen without gonads appear in grafts of the spayed female series giving a ratio of twenty-four testes to twenty-nine ovaries exhibiting various degrees of differentiation. Gonad differentiation, therefore, parallels the earlier experiment. Ovarian development is inhibited, as reported previously by Buyse ('35). This inhibition is due to adverse mechanical conditions in the graft environment since well-formed ovaries are usually found in coelom-like spaces where growth appears less restricted. These experiments demonstrate that the sex hormones of the adult rat play no role in the differentiation of fetal rat gonad primordia since the several types of grafts recovered in normal and gonadectomized hosts of either sex are identical.

Physiology

152. THE ACTIVATION AND DEPRESSION OF YEAST RESPIRATION BY RADIATIONS. John C. Fardon, Robert J. Norris and Sister M. Veronita Ruddy, Institutum Divi Thomae. (Introduced by W. C. Curtis.)

The irradiation of cells by ultraviolet has failed to give consistent results in its effect on fermentation. Contradictory claims are made by various workers including Tanner and the De Fazis.

These same inconsistent results were encountered in investigations on the influence of irradiation on the respiration of yeast. The respiration increased in some cases and in others decreased with length of exposure to ultraviolet or x-rays.

Numerous possible causes for this variation were investigated. The age of the yeast was not the factor; changes in pH from 6.9 to 7.8 did not affect the O_2 consumption. Many possibilities were studied but neither the spectral distribution nor any other single factor was totally responsible for the inconsistent results.

These studies leading to no solution, the following hypothesis was made: Two factors are responsible for the change of respiration, 1) cells are destroyed in varying porportion according to the length of exposure, and dead cells do not respire; and 2) either the dead or dying cells secrete a substance, or the ultraviolet 'activates' a compound, which has the power to stimulate the remaining living cells. The varying proportion of these factors is the reason for the varying phenomena of increase or decrease in respiration.

Evidence for the existence of this second factor was obtained when yeast suspensions, irradiated until they showed no respiration, were added to non-irradiated cells, and there at once resulted a marked increase in respiration of the living cells.

153. THE PROLONGATION OF LIFE IN ARBACIA EGG CELLS. Victor Schechter, College of the City of New York.

The unfertilized egg cells of Arbacia punctulata have a very definite life span. In sea water at 21°C. some begin to disintegrate in 12 to 15 hours. Death then occurs rapidly. In about 30 hours half are dead and all have disintegrated 20 hours later. The measure of death used was cytolysis; or irregularity in shape as an indicator of loss of semipermeability. Supplementary fertilization and cleavage tests were made.

Decreasing the normal calcium concentration in sea water prolongs life in these cells far beyond the usual. When calcium concentration was halved 50% of the eggs liver 50 hours; with one-third calcium over 60 hours; with one-sixth calcium almost 80 hours; with one-twelfth calcium 90 hours. The effect was due in part to increased acidity in the artificial solutions. When both Ca' and H' varied the life span was tripled. With constant pH the duration of life was doubled.

The effect herein reported is of interest not only with regard to the mechanism of ageing but also in relation to the evolutionary problems of the time of origin of Echinoderms and adaptation to changes in salt concentration in the sea.

154. TEMPERATURE DEPENDENCE OF METABOLIC CHANGE ON FERTILIZATION IN CHAETOPTERUS EGGS. Herbert Shapiro, Princeton University.

The eggs of the worm, Chaetopterus, and of the mollusc Cumingia, differ in their metabolic phenomena from other marine eggs hitherto investigated in showing a decided drop in rate of oxygen uptake upon fertilization—about 50% at 21°C. (Whitaker, '33). According to Rubenstein and Gerard ('34) the increase of respiration of Arbacia eggs on fertilization depends upon the temperature at which the changes are measured, being approximately 7: 1 at 14.3°, and only 2: 1 at 29.3°. Using a differential volumetric microrespirometer, where the eggs were not shaken, the temperature dependence of oxygen uptake on fertilization was examined in Chaetopterus eggs. The finding of Whitaker was confirmed qualitatively, and it was also found that the temperature effect is not as pronounced as in Arbacia. The drop in respiration was approximately 60% at 10°, but became less with elevation of temperature, being only about 20% at 31°. The Q_{10} of resting eggs was lower than that of fertilized, which is the reverse of the situation described for Arbacia eggs, where respiratory changes on fertilization are likewise in the opposite direction. Possible explanations of these phenomena are a shift on fertilization in substrate-enzyme relations, or an alteration in the respiratory mechanism, by which the respiration is transferred from one enzyme system to another, whose temperature coefficient of activity is different.

155. A STUDY OF THE PROCESS OF FISSION IN THE NAID, PRISTINA LONGISETA. C. D. Van Cleave, University of Pennsylvania.

Mass cultures of Pristina longiseta were grown in the laboratory in protozoan cultures based on boiled wheat. Statistics are given to show the variation in number of segments and of zooids in the individuals constituting the population under various conditions of food supply and temperature and at various periods in the life cycle of the culture. It is concluded that the position of the fission zone can be influenced by such factors as nutrition, temperature and age of culture. Conditions favorable to the rapid growth of new segments, such as ample food, temperature of 20 to 25°C. and fresh culture medium, produce intense fissioning activity in the course of which the fission zones shift anteriorly. With poor nutrition at the same temperature the fission zone shifts posteriorly, giving long zooids. With good nutrition at a temperature of 10°C. the fission zone shifts anteriorly, giving very short zooids. Worms grown in previously 'used' or conditioned water at 20 to 25°C. and with ample food had very long zooids. The process of fission is regarded as an instance of physiological isolation and consequently the environmental factors as influencing its position by altering the range of dominance of the head end of the zooid.

156. FURTHER EXPERIMENTS ON POSTERIOR REGENERATION AND THE NERVE CORD IN THE EARTHWORM EISENIA. P. L. Bailey, Jr., Marine Biological Laboratory, Woods Hole, and the College of the City of New York. (Introduced by R. C. McCardle.)

The cord was cut transversely, posterior to the clitellum, and the anterior piece looped forward as described in the author's previous experiments. In other cases a piece of cord from the clitellar region was drawn forward without looping and allowed to grow alongside of and parallel to the old cord. Later a transverse cut was made across the entire animal in the region of the double cord, leaving two ends of cord tissue exposed at the cut surfaces. If the cord influences regeneration, as some believe, it might be expected that supernumerary or abnormal tails would arise on the anterior piece. Of individuals surviving the second operation, one-half regenerated normal tails, thus showing no special influence of the extra nervous tissue. The others developed slightly abnormal external scar formations, or blunt wound closure without regeneration. The latter showed no histological evidence of any nervous tissue near the healed region. It is possible that this tissue contracted anteriorly from the cut surface at the second operation, and complete healing over of the wound before the cord could regenerate to the cut surface prevented the cord from exerting any initiating influence on the regeneration of a new tail. Histological examination shows, with but one exception, no evidence of the isolated nerve tissue, indicating that it probably degenerated. This may also indicate that isolated nervous tissue transplanted from its normal position cannot influence regeneration.

157. PRODUCTION OF ACCESSORY TAILS IN THE EARTHWORM BY POSTERIOR BISECTION.

P. L. Bailey, Jr., Marine Biological Laboratory, Woods Hole, and the College of the City of New York. (Introduced by R. C. McCardle.)

The posterior ends of earthworms (Eisenia) were splite longitudinally for several segments and allowed to heal and regenerate. In some specimens the posterior twenty to forty segments were amputated before the split. In no case did the cut extend as far forward as the clitellum. In two individuals, healing of one of the split halves occurred resulting in a functional tail. The other half partly sloughed off and healed without forming a new tail. However, in both cases, regenerating segments which proved to be functional tails appeared at the anterior apex of the bisection. Histological examination showed that the gut, nerve cord, and blood vessels had bifurcated at the region where the new tail was forming, and the segments of the new part appeared to be entirely normal, though smaller than those in the old part. These individuals are evidence that supernumerary structures need not necessarily be always the result of developmental anomalies or grafting processes as some investigators have believed, but may occur as well during adult life through experimental or accidental means. Just what initiates the production of the accessory tail is not clear. In all cases gut, blood vessels, and nerve cord had been injured in the operation.

158. ELECTRICAL INHIBITION IN THE HEART OF THE OYSTER.

Edward M. Walzl, Johns Hopkins University.

The question has frequently arisen as to whether inhibition of cardiac and smooth muscle, resulting from electrical stimulation, is always dependent on the presence of nerves.

The following observations were made in a study of the response of the excised heart of the oyster to electrical stimulation.

1) The response is not 'all or nothing.' 2) The absolute refractory period is very short, since response is obtainable during almost all phases of contraction and relaxation. 3) The nature of the response depends on the phase of contraction

the heart is in when it is stimulated, i.e., single condenser discharges applied during the period from the end of diastole to half systole cause extra systoles, while those applied at any other time cause relaxation. 4) Short faradic stimulation causes in all phases relaxation which is followed by contracture. Stimulation during the contracture causes momentary relaxation. 5) Prolonged faradic stimulation causes immediate relaxation followed by resumption of a slow rhythmic beat of small amplitude. Cessation of stimulation at any time results in immediate contracture. 6) Only cathodal stimulation is effective in producing relaxation. 7) Strength-duration curves for electrical stimulation are unbroken and have a slope of —1 for short durations. 8) Sympathetic and parasympathetic nerve poisons do not affect this response.

The above observations show that the heart of the oyster resembles smooth muscle in its response to electrical stimulation, and that its highly developed inhibitory response is an inherent property of this muscle, probably not referable to the action of nerves.

159. EFFECT OF CALCIUM AND POTASSIUM ON THE THRESHOLD OF EXCITATION OF THE HEART OF THE OYSTER. Edward M. Walzl, Johns Hopkins University.

In previous work on the heart of the oyster it was concluded that excess calcium acts mainly by inhibiting the excitatory mechanism, and excess potassium, by inhibiting the contractile mechanism.

In further observations on this heart perfused with, 1) sea water, 2) sea water plus calcium chloride, and 3) sea water plus potassium chloride the following results were obtained.

Concentrations of calcium chloride sufficient to decrease the frequency of contraction 50% and increase the amplitude of contraction 10%, raised the threshold for electrical stimulation by approximately 100%.

Concentrations of potassium chloride sufficient to decrease the amplitude of contraction 50%, had little effect on frequency of contraction, and no observable effect on the threshold of excitation.

These results confirm the previous conclusions.

160. SEASONAL WEIGHT AND TOTAL WATER CONTENT OF THE MOUND-BUILDING ANT, FORMICA EXSECTOIDES, FOREL. William A. Dreyer, University of Cincinnati.

The purpose of this investigation was to determine the relation of body weight and total water content to temperature and the seasonal fluctuation of metabolic activity previously described for the mound-building ant. Body weight of workers varies from 6.4 mg. to 17.5 mg., mean 11.65 mg. Ninety-one and one-tenth per cent of the determinations occur between 9 mg. and 15 mg. Total water content varies from 62.1% to 81.6% of the live body weight. The mean is 72.7%. Ninety-one and six-tenths per cent of these determinations occur between 68% and 78% of the live body weight.

There is no significant variation in body water content in relation to weight within the range of the experiments. Neither is there any significant difference in the water content of individuals from one colony compared to the water content of those from another colony, nor of animals kept in the laboratory from 11 to 27 weeks compared to those recently collected. The total water content of active animals in summer, hibernating animals in winter, and animals subjected to seasonal reversal or extension of activity and hibernation varies within the range stated. There are no significant differences occurring between any of the normal or experimental conditions.

Seasonal fluctuation of total water content is not a factor in the alteration of metabolism occurring during the hibernation of this species. The observations indicate the existence of an adaptive relationship when compared with similar observations upon other insects.

161. THE REPUGNATORIAL GLANDS OF ONCHIDIUM. Leslie B. Arey, Northwestern University Medical School.

There are about six chief glands and three accessory glands buried in the mantle fold of each side. A robust muscular sheath surrounds each chief gland. The gland cells are club-shaped elements up to 350 μ long and are at least of two different kinds. One is highly luminous by reflected light and stains with osmic acid; others have a granular content. Cytological details will be given at a later time.

The gland discharges after elevation of the mantle fold and the erection and direction of a prominent papilla which bears the duct. A fine stream of viscid fluid is shot out to a distance up to 15 cm. In nature mechanical insult is the adequate stimulus; this does not act by local application but from a distance. In addition the faradic current stimulates when applied locally. The nervous transmission of distant stimuli can be blocked with novocaine without interfering with the responsiveness of the glands themselves. Most important of the chemical stimulants is sodium chloride in the same concentration that it occurs in sea water. It can be proved that the cation sodium is the responsible agent and that its effect is completely antagonized by magnesium, also in the same concentration as in sea water.

The secretion is clear by transmitted light but milk white by reflected light. Its fluid base contains dense, fine granules and sparser oil globules. The secretion stings the tongue because of its oil content. The granular mass is responsible for an acidity of pH 3.0, yet the secretion does not taste sour.

162. ELECTRICAL VARIATIONS IN THE HEART OF THE ADULT GRASSHOPPER (MELANOPLUS DIFFERENTIALIS). Theodore Louis Jahn, Frederick Crescitelli and Aubrey Bryant Taylor, State University of Iowa.

Photographic records of the electric variations associated with the beating heart of the adult grasshopper (Melanoplus differentialis) have been made, utilizing a three-stage condenser-coupled amplifier and a cathode ray oscillograph. Silver-silver chloride capillary electrodes have been applied either to the dorsal or ventral surface of the intact heart. When both electrodes are placed along the longitudinal axis of the heart each beat is recorded as a diphasic potential variation each phase of which is composed of a single peak or of several partially superimposed peaks. The general form of the wave is unaffected by variations in the interelectrode distance. The potential variation is such as to suggest an anterior progression of the excitatory state from the caudal region. In some cases the records indicate a reversal in the direction of propagation, and in accordance with this finding a reversal in the direction of the wave of contraction has also been noted. A diphasic wave form is also obtained when all parts of the heart appear to be beating simultaneously.

The records also demonstrate the existence of diphasic potential variations with a frequency about six to ten times that of the wave associated with the heart beat. These high frequency variations, which are present in most but not all records, appear to have no definite temporal relation to the contractile process and often continue when the latter is temporarily inhibited by low temperature. The origin and significance of these secondary potentials has not been determined.

163. RESISTANCE AND CAPACITANCE MEASUREMENTS ON GRASSHOPPER EGG MEMBRANES OVER A WIDE FREQUENCY RANGE. Kenneth S. Cole and Theodore Louis Jahn, College of Physicians and Surgeons, Columbia University, State University of Iowa and Biological Laboratory, Cold Spring Harbor.

Measurements have been made of the parallel resistance and capacitance of Melanoplus differentialis egg membranes, both before and after the formation of the cuticle, over the frequency range 0.03 to 2500 kilocycles, in sea water, in dilute sea water, and in 1 N and 3 N KCl.

The chorion of pre-cuticular membranes is equivalent to a polarization impedance and a resistance in parallel. The impedance is independent of the medium and has a capacitance of 0.027 μ f/cm.^{2} at 1 kilocycle, with a phase angle of 81°, while the resistance and consequently the characteristic frequency depends upon the specific resistance of the medium. The membrane has an approximate cell constant of 5.0 for 1 cm.^{2}

The membranes of late diapause eggs (which contain both chorion and cuticular layers) are nearly equivalent to a polarization impedance and resistance in parallel. The impedance is independent of the medium and has a capacitance of 0.1 μ f/cm.^{2} at 1 kilocycle with a phase angle of 85°. The resistance is approximately 45,000 ohms/cm.^{2} and is independent of the medium. The characteristic frequency has a value of about 30 cycles. There are indications of a second variable impedance element effective at high frequencies. No evidence for the elctrical properties of the chorion was found in the late diapause membranes where both chorion and cuticular layers were present. Therefore, a modification of these properties is indicated.

164. A BIOLOGICAL TEST OF THE INVERSE SQUARE LAW OF RADIANT ENERGY AS APPLIED TO ROENTGEN RADIATION. H. Dabney Kerr and Titus C. Evans, State University of Iowa and A. and M. College of Texas.

Eggs of Melanoplus differentialis and of M. bivitattus have been used as test materials in comparing the loss in biological effectiveness with loss in dosage and intensity as the target distance is increased. It has been found that in a restricted range the loss of biological effect parallels the decrease in dosage and intensity (which follows the inverse square law).

Variations of biological effects from the theoretical have been noted as follows: 1) In all cases the biological loss is less than the theoretical for increases in distance of only a few centimeters from the shortest distance employed, and the loss is greater than indicated by the inverse square law when the increase is extensive. 2) The sensitivity of the eggs affects the character of the curve. 3) Intensity is also a factor, and 4) preliminary experiments indicate that recovery accounts for only a part of the intensity effects.

165. ULTRAVIOLET IRRADIATION OF GRASSHOPPER EMBRYOS (MELANOPLUS DIFFERENTIALIS). O. Malcolm Ray, State University of Iowa. (Introduced by H. W. Beams.)

Experiments 1, 2 and 3 consisted of the irradiation of eggs for 1 hour, 2 hours and 4 hours daily from the beginning of development after diapause until hatching (18 to 21 days). In the first two experiments the control and experimental animals began hatching on the same day and the oxygen consumptions were very similar, indicating no effect of the ultraviolet irradiation. In the third experiment the eggs began to hatch on the same day but only 80% of them hatched.

In experiments 4, 5 and 6 developing eggs with the chorion removed were irradiated 4 hours, 2 hours and 30 minutes daily in the respective experiments. In all three cases there was a slight increase in the oxygen consumption during the first 4 days and then it decreased to below the beginning value and the embryos died in 10 or 12 days. This indicates that the chorion acts as an effective shield against ultraviolet light.

In experiment 7 an area of the egg that did not expose the embryo was dechorionated and irradiated for 30 minutes daily. About the eighth day during yolk engulfment the embryo appears in this area and the fact that there is a decreased oxygen consumption only when the embryo is not covered by the chorion indicates that the effect is due to direct action on the embryo and not to secondary irradiation of the egg or yolk. It also testifies to the protective nature of the chorion.

166. RESISTANCE OF GRASSHOPPER EMBRYOS TO HYPERTONIC SOLUTIONS. Arthur Louis Schipper, State University of Iowa. (Introduced by H. W. Beams.)

Eggs of the common roadside grasshopper, Melanoplus differentialis, containing embryos of selected morphological stages, were placed in aerated hypertonic balanced salt solutions for a definite period and then transferred to an aerated isotonic balanced salt solution. The ability of the embryos to resume normal development, subsequent to such treatment, was used as an index of the resistance of the embryos. Of the prediapause embryos it was found that the least resistant stage occurred just at the time when a definite embryo was being formed. Stages preceding and succeeding were more resistant, this resistance increasing until a maximum is reached when the embryo enters diapause. Of the postdiapause embryos the least resistance was noted during the process of blastokinesis. Previous to blastokinesis the diapause condition was simulated. Stages following blastokinesis show an increase in resistance but did not show the marked difference noted in the prediapause embryos. There is an indication that short exposures to those concentrations which retard (but do not inhibit) postdiapause development are more injurious than equal exposures to those concentrations which inhibit development. Thus it is apparent that active cells are less resistant than cells which are inactive even though this inactivity is brought about by the removal of water from the cells.

167. EFFECT OF DINITROPHENOLS ON RESPIRATION OF GRASSHOPPER EMBRYOS.

Joseph Hall Bodine and Edgar J. Boell, State University of Iowa.

When 2,4-dinitrophenol (DNP) or 3,5-dinitro-o-cresol (DOC) in suitable concentrations are added to isolated embryos of the grasshopper, Melanoplus differentialis, an immediate increase in the oxygen uptake of the embryos is noted. In the case of blocked embryos, or those in diapause, the increase amounts to approximately 350% of the normal; with actively developing embryos the respiratory rate in the presence of the nitrophenols is about 250% of the control values. The absolute value of O_2 uptake in the case of diapause embryos plus DNP or DOC is lower than that of developing embryos plus these substances since the normal respiration of diapause embryos is only one-third as large as that of developing embryos. Maximum stimulation with DNP is obtained with a concentration of ca. 2×10^{-5} molar. With DOC the optimal concentration is somewhat lower.

The stimulation of O_2 uptake is practically completely prevented by carbon monoxide. Moreover, in developing embryos, CO reduces the respiratory level to less than the normal even in the presence of DNP or DOC. Cyanide, when added to embryos before the DNP or DOC are added, can reduce stimulation, prevent it entirely, or reduce O_2 uptake below the normal depending upon the concentration of cyanide used. The same level of respiration is reached whether cyanide is added before or after DNP or DOC or simultaneously with it. The increased respiration has a Q_{10} approximately the same as that of the normal respiration, viz., 2.0.

168. EFFECT OF TEMPERATURE UPON THE 'BLOCK-MECHANISM' (DIAPAUSE) OF THE GRASSHOPPER EGG, M. DIFFERENTIALIS.

J. H. Bodine, State University of Iowa.

Eggs of known history have been subjected, at different ages, to the action of constant low temperatures (0°–5°–10°–15°C.). The effects of these temperatures on the destruction or inactivation of the block-mechanism or diapause have been studied by following their subsequent development at 25°C. Rates of O_2 uptake have also been determined in order to follow physiological changes occurring in the eggs.

Temperatures of 10°C. and above not only destroy the potency of the block but also permit of some development. In such eggs where destruction of the block and embryological development occur simultaneously, the low temperature is most efficient in destroying the block. At constant low temperatures (0° to 5°C.), on the other hand, the block is destroyed but at a relatively slower rate than at a temperature high enough for development but at the same time destructive to the block or inhibitory mechanism. Results of these observations seem to show that close relations between developmental activity and the potency of the block-mechanism exist and thus make possible a further and more detailed analysis of the action and interaction of each.

169. EFFECT OF CYANIDE ON RESPIRATION OF MELANOPLUS DIFFERENTIALIS EGGS.

Wilbur Robbie, State University of Iowa. (Introduced by J. H. Bodine.)

A study has been made of the effect of various concentrations of cyanide on the O_2 uptake of the eggs of Melanoplus differentialis by means of the Barcroft-Warburg apparatus at temperatures ranging from 15° to 35°C. Cyanide solutions were prepared by neutralizing KCN with HCl and diluting with M/100 phosphate, pH 7.0, to the concentration desired (0.0001 to 0.01 N) immediately before use. KCN-KOH mixtures having the same HCN tension as that of the solution surrounding the eggs were placed in the center wells of the manometer flasks to absorb CO_2.

The results of preliminary experiments show that eggs are more sensitive to KCN during the post-diapause stage of development than in the diapause or early pre-diapause periods, and that sensitivity during the post-diapause period does not change markedly. Nearly the maximum effect of cyanide on respiration was noted within a few minutes after the start of the exposure, but recovery on removing the eggs from the KCN solution required several hours, or even days with the higher concentrations.

Although 0.01 N KCN reduces respiration to less than 10% of the normal and may prolong the time of hatching several days, the fact that more than 50% of the eggs thus treated may hatch shows that exposure at this concentration is not beyond the limit of reversibility. At 30°C. the percentage depression caused by KCN is greater than at 25°C. Existence of different races (?) of eggs, varying in their normal oxygen uptake and in their sensitivity to KCN has been noted.

170. THE EFFECTS OF HYPO- AND HYPERTONIC SOLUTIONS ON THE TISSUES OF FISH.

R. T. Young, Scripps Institute.

A large number of experiments with both hypo- and hypertonic solutions on various species of marine and fresh water fish indicate that: 1) No consistent pathological effect can be ascribed to them. 2) When pathological effects are evident they are probably secondary, and not primary causes of death, and may possibly be attributed to some weakness of the specimens which show them. 3) Respiratory disturbance of some sort is probably the primary cause of death; but this may, or may not be accompanied by pathological changes in the gills themselves.

171. THE EFFECT OF BALANCED AND UNBALANCED SOLUTIONS, OF pH AND DISTILLED WATER ON FISH.

R. T. Young, Scripps Institute.

A large number of experiments have been performed to test the effect of these factors on both marine and fresh water fish, including species of Embiotocidae, Fundulus parvipinnis and Apomotis cyanellus. For the marine species balanced solutions were made by the addition of Na, K, Ca and Mg chlorides and $MgSO_4$ in the proper proportions to distilled or to sea water, while unbalanced solutions were prepared by addition of these salts singly and in various combinations to distilled water, sea water and various combinations thereof. For Apomotis balanced solutions were obtained by adding sea water to lake water, and unbalanced solutions by the addition of NaCl to distilled or to lake water.

In testing the effect of distilled water both singly and doubly distilled water has been used.

The results of these experiments indicate that: 1) Osmosis is a more important factor in the lives of fish than is exact balance of ions, which is of less importance than is generally assumed, but 2) A certain degree of balance is essential. 3) Two ions as nearly related chemically as Na and K, for instance, may exert very different physiological effects. 4) No conclusions can yet be drawn as to how ions exert their effect, 5) Certain fishes, at least, can withstand a wide range in pH. 6) Distilled water (either singly or doubly distilled) is fatal to some fishes at least. 7) pH plays only a minor, if any part in the toxicity of distilled water.

172. The rate of adaptation of Fundulus in sea water to black and to white backgrounds at different temperatures. William H. Cole and K. F. Schaeffer, Rutgers University.

The time for complete melanophore adaptation of intact Fundulus heteroclitus in sea water to black and to white backgrounds has been measured at seven temperatures from 8° to 28°C. At all temperatures the time for adaptation to a white background is less than that for adaptation to a black background, but the temperature characteristics calculated from the Arrhenius equation appear to be the same. For dispersion of melanin within the chromatophores (or adaptation to black background) the upper critical temperature is near 25°, above which the fish tend to remain light. This is the well-known high temperature effect on melanophores, i.e., concentration. The lower critical temperature for dispersion is near 11°, below which the well-known 'cold block' increases the time for adaptation. For concentration of melanin (or adaptation to white background) no upper critical temperature appeared, probably because high temperatures facilitate concentration. The lower critical temperature for concentration is less than 11°, below which the fish tend to remain dark due to cold block. Between 11° and 25°, therefore, increase in temperature causes increased rates of adaptation to black and to white backgrounds, but the latter (concentration) averages about 2.8 times faster than the former (dispersion). Below 11° and above 25° rates of adaptation vary according to the direction of adaptation. Melanophores of intact Fundulus respond to background stimuli by two different mechanisms depending upon the temperature range.

173. An independent response to light on the part of the scale melanophores of Tautoga onitis (L.). Dietrich C. Smith, University of Tennessee College of Medicine.

The melanophore pigment in the isolated scales of Tautoga onitis will show a brief centrifugal migration when exposed to a sudden increase in light intensity, followed by a return to its former condition. Scales demonstrating such responses were taken from the trunk region of the fish and placed in an M/5 solution of the following salts mixed according to the proportions recommended by Spaeth; NaCl 6 vol., KCl 1 vol., and $CaCl_2$ 0.35 vol. The melanophore pigment in such a solution assumes a state of almost complete concentration. The scales were illuminated by a 165-watt microscope lamp placed about 2 feet from the substage mirror. The experiments were performed at night in a dimly illuminated room. The illumination on the scales was varied by turning the lamp off and on. After at least 10 seconds in darkness dispersion of the melanophore pigment immediately followed turning on the light. The amount of dispersion was within limits proportional to the time the light was off. The best responses were obtained when the time in darkness exceeded 1 minute. The centrifugal migration began immediately on re-illumination and in 30 seconds the pigment reached a state of complete dispersion. At this point the direction of the movement was reversed and within 5 to 6 minutes the pigment had reverted to its former condition of semi-concentration.

174. THE EFFECT OF LIGHT ON SEXUAL ACTIVITY IN THE LIZARD, ANOLIS CAROLINENSIS. H. J. Clausen and E. G. Poris, American Museum of Natural History.

Two groups of adult male lizards were subjected daily to 6 hours additional light from 80-watt incandescent bulbs over a period of 50 days beginning November 25th. Lizards in one of the above groups (sixty animals) were kept as normals while the sixty lizards in the other group were pinealectomized. As a control, two groups of thirty-five lizards each received no added light. Here again one group was pinealectomized while the other group was retained as normals.

All lizards from the four groups were killed on January 25th and a microscopic study made of the testes. The results show that while testis activity in the normal controls was progressive during this season, the added illumination served to stimulate activity so that complete spermatogenesis was evident in a large percentage of cases. The results also indicate that the removal of the pineal further increases testis activity in either the control group or the group receiving added illumination. Hence, the pineal does not act as a primary light receptor for sexual stimulation. These experiments suggest that the pineal either acts as an inhibiting receptor or it produces a secretion having those inhibiting properties.

175. THE PINEAL OF THE LIZARD ANOLIS CAROLINENSIS, A PHOTORECEPTOR AS REVEALED BY OXYGEN CONSUMPTION STUDIES. H. J. Clausen and B. Mofshin, American Museum of Natural History.

Oxygen consumption rates of a series of lizards (Anolis carolinensis) were recorded, using a slight modification of the Haldane-Henderson gas analysis apparatus. Tests were carried out at a constant temperature of 30°C. Comparative tests were made in illuminated and in dark surroundings. Normal lizards and lizards having one or more receptors incapacitated were used in the tests. Hourly readings were made over a 3-hour period for each individual series.

On the basis of differences obtained in the rates of oxygen consumption under the dark and the light condition, the results indicate the eyes as being of essential importance as light receptors. Nevertheless, the skin was shown to be approximately equal to the eyes in this respect. Furthermore, while the pineal body proved to be of less importance as a receptor, a statistical analysis of the data indicates that this organ functions as an agency for the perception of light.

176. Cheyne-Stokes respiration; a study in the control of rhythm. F. H. Pike, Columbia University.

Attempts to reduce the control of normal respiration to one factor have been unsuccessful. The demonstration of independent excitability of the central nervous mechanism for respiration is a necessary, but not a sufficient, condition. Afferent nervous impulses enter into the control of all respiratory movements in vertebrates, and probably some invertebrates, under normal conditions. The equation

$$R = f(D)(V)(T)(N),$$

in which respiratory rhythm R is some function of substances (D) dissolved in the blood, (V) the volume and (T) the temperature of the blood flowing through the central mechanism in unit time, and (N) afferent nervous impulses, the variables not being independent, represents a truer conception.

Cheyne-Stokes respiration, seen experimentally as well as clinically, is irregular, the slowest rhythm coming when the concentration of CO_2 is highest, and conversely. Changes in blood gases alone cannot explain it.

The hypothesis of changing conductivity of central nervous pathways explains these, and other, facts better. With gradually failing bodily functions, decrease in central conductivity would leave the central respiratory mechanism dependent upon blood gases alone for excitation, with slow rhythm. Slight changes in central conditions determine whether the same central mechanism responds to afferent impulses reaching it over contralateral conduction paths, or ipsilateral only. Slight change for the better, with increased O_2 and decreased CO_2, would account for brief periods of higher rhythm.

177. Effect of splenectomy on thyroxine tolerance. Albert S. Gordon and William Kleinberg, Washington Square College, New York University.

Bodansky and Duff have demonstrated a tolerance of young rats to daily 1 mg. injections of thyroxine for over a period of 1½ months. Similarly, we have found that young normal and operated control hooded rats (30 to 60 days) will tolerate daily injections of 2 mg. of crystalline thyroxine for longer than 2 months. Splenectomized rats, however, of the same age, when injected daily with doses of either 1 or 2 mg. of thyroxine, die within 3 weeks. All the rats used were free from bacterial infections, such as Bartonella muris, for which they were carefully examined throughout the entire course of the investigation. When subjected to daily 1 mg. injections of thyroxine, young splenectomized guinea pigs were found to succumb more quickly than normal and operated litter mate controls.

Work is now in progress dealing with the relation of the spleen to the development of refractoriness to various endocrine principles.

178. LONGEVITY OF ERYTHROCYTE AND RETICULOCYTE IN NORMAL AND SPLENECTOMIZED GUINEA PIGS. Albert S. Gordon and William Kleinberg, Washington Square College, New York University.

We have employed the method of Escobar and Baldwin to determine the longevity of the guinea pig erythrocyte. This consists in subjecting the animals to pressures sufficiently low to result in a significant increase in red cell count, and then recording the time for the return of the red cell count to its original level. Whereas the red cells of normal and operated control guinea pigs showed a longevity ranging from 20 to 29 days, the red cell longevity of splenectomized animals was observed to range from 29 to 37 days. This greater longevity of the red cell in splenectomized animals is attributed to 1) the removal, with the spleen, of a large portion of the reticulo-endothelial system normally concerned with red cell phagocytosis and destruction and 2) the increased resistance of the red cells following splenectomy.

By observing the time necessary for the reticulocytes to reach normal values after the termination of the low pressure stimulus, we have found the longevity of the circulating reticulocyte to be unaffected by splenectomy. It ranges from 4 to 6 days in all the splenectomized and normal animals.

179. A BRAIN DISORDER IN YOUNG CHICKS FED ON A DIET TREATED WITH FERRIC CHLORIDE FOR THE DESTRUCTION OF VITAMIN E. F. B. Adamstone, University of Illinois.

Newly hatched chicks which have been fed continuously on a natural diet treated with ferric chloride in ether to destroy vitamin E develop a characteristic nervous disorder. The condition appears suddenly about the fourth week (sometimes later) and affects about one-third of the chicks. It is first recognizable as an uncertainty of gait. This is followed by staggering and finally by prostration accompanied by spasticity in the legs and occasionally, head retraction. Spontaneous recovery sometimes occurs but the condition is usually fatal.

The cause of the condition is traceable to degeneration in the brain particularly the cerebellum and rarely the cerebrum. Edema and hemorrhage are common. Histological studies of the cerebellum show disorganization of the outer gray layer, disintegration of the Purkinje cells of the ganglionic layer marked by changes in staining reaction, loss of Nissl substance and swelling of nuclei. The cells of the inner granular layer become strongly pycnotic. In the medulla the fibers degenerate and large holes are formed.

Attempts to prevent the occurrence of the condition by feeding wheat germ oil and highly concentrated extracts of vitamin E have not been successful and the inclusion of lard in the diet (treated or synthetic) has been very harmful.

The condition appears to be identical to that described by Pappenheimer et al. and this work would appear to support his conclusion that vitamin E deficiency is not alone responsible for the condition.

180. SYNTHESIS OF VITAMIN C. C. P. Leblond, Yale University. (Introduced by Edgar Allen.)

Most animal species synthesize enough vitamin C to live normally without receiving it in the diet. Under these conditions the acid silver nitrate reaction, which is specific for indicating the presence of the vitamin, may be observed in the organs and tissues.

However, on a vitamin C free-diet man, monkey and guinea pig die of scurvy because they either synthesize too little or none of the vitamin. During pregnancy, however, the scurvy is delayed until after parturition and therefore either mother or the embryo or both produce the vitamin.

In order to determine the role of the maternal organism, Loeb's experiment was utilized. Conditions of pseudo-pregnancy in the guinea pig were produced by ablation of the uterus several days after estrus was established. The animals were then given a vitamin free diet. The pseudo-pregnancy, confirmed by persistence of the corpus luteum and absence of the opening of the vagina, did not delay the onset of scurvy.

The probable role of the fetus has been confirmed by observations on pregnant guinea pigs during the second part of pregnancy. When given a vitamin free diet, silver nitrate reactions in the organs of the fetus could be found, while the organs of the mother did not react.

The place of the synthesis could not be determined. The ablation of hypophysis, genital or thyroid glands did not hinder the synthesis in the rat, since the organs of rats thus operated and given a vitamin free diet, continued to give the silver nitrate reaction.

181. Light in its relation to activity and estrus rhythms in the albino rat.

Ludvig G. Browman, The University of Chicago.

Mature female rats placed in automatically recording activity cages were exposed to light from ordinary electric light bulbs for varying periods of time. The peak of daily activity during normal day-night or during controlled 12-hour artificial light and 12-hour darkness occurred at night or during darkness. Reversal of the 12-hour light conditions caused a subsequent reversal of daily activity. Reversal of light conditions had no detectable effects on the estrous cycles of any of the animals.

Constant artificial light for a period of weeks induced fluctuations in the daily activity rhythms of female rats. Thus, peaks of daily activity might occur for 5 to 6 days during the solar night, then 1 to 3 days of arrhythmia, followed by 5 to 6 days with the peak of daily activity occurring during the solar day, and so on. This fluctuation of daily activity rhythms continued as long as the animals were exposed to continuous light. Prolonged and constant vaginal cornification ('light estrus') occurred in over one-half of the animals exposed to continuous artificial illumination.

Animals in constant darkness, and animals that had been made blind, maintained constant daily activity rhythms with normal estrous cycles. Animals in continuous darkness tended to maintain the daily activity rhythm with which they entered the darkness. Thus, animals from continuous light with peaks of activity during the solar day continued unchanged in this rhythm when placed in the dark. Neighbor animals with exactly opposite rhythms continued their different daily rhythms without influencing or being influenced by the activity rhythms of other animals.

No significant differences in the total activity (number of revolutions) of rats under the different light conditions was observed.

Endocrinology

182. THE CHROMATOPHOROTROPIC HORMONES. A. A. Abramowitz, Harvard University. (Introduced by F. L. Hisaw.)

The chromatophorotropic hormone obtained from the crustacean eyestalk was standardized on eyestalk-extirpated crabs, Uca pugilator and Uca pugnax. Duration of response (melanophore expansion) was exponentially proportional to concentration of hormone. The hormone content in the eyestalks of various other crustaceans was determined in terms of Uca equivalents. The active principle was soluble in methanol, ethanol; slightly soluble in acetone; insoluble in benzine, chloroform, ether; thermostable, destroyed by oxidation, stabile to acids and alkali. Some purification was accomplished by precipitation with ether of the alcohol or acetone (95%) soluble portion, and repeated washings with chloroform to remove a red carotenoid pigment. The final solution was water clear and protein free.

Eyestalk extracts of any of thirty crustacean species produce when injected into appropriately prepared Uca, Palaemonetes, and Crago the same qualitative response of the various types of chromatophores as produced by extracts of these test animals' own eyestalks. Since the chromatophoral organization of these animals is different anatomically and physiologically it appears that the nature of the chromatophoral response to the hormone is determined by the pigmentary organization of the animal, not by the hormone.

The invertebrate hormone expands melanophores of hypophysectomized vertebrates (amphibian, teleost, elasmobranch, reptile); the vertebrate hypophyseal hormone (sheep Intermedin) expands melanophores in eyestalk-extirpated invertebrates (Brachyura). The known chemical properties of both vertebrate and invertebrate pigmentary hormones are also closely similar. Furthermore, extracts of the ascidian (Molgula) subneural gland were found to contain the chromatophorotropic hormone (0.4 Uca equivalents).

183. A COMPARATIVE STUDY OF WATER METABOLISM IN AMPHIBIANS INJECTED WITH PITUITRIN. F. R. Steggerda, University of Illinois.

Since it has been established that frogs (Rana pipiens) injected with pituitrin (0.1 cc. per 10 gm. weight) increase approximately 18% in weight due to the absorption of water, it was of interest to compare the difference in weight changes in different amphibia when treated in a similar way. The animals selected were the toad (Bufo americanus), mud puppy (Necturus maculatus), and two species of frogs (Rana pipiens and Rana clamitans).

The procedure was to nearly submerge the animals in tap water at room temperature for at least 12 hours before injecting with pituitrin subcutaneously. After the injection the animals were returned to the water and routine weighings were made at ½-hour intervals for 7 to 10 hours. The percentage difference in weight from the control weight taken before the injection was then calculated and plotted.

The results show that the injection of pituitrin into the animals gave the following average increase in weight: nine toads, 45%; six Rana pipiens, 18%; six Rana clamitans, 14%; six Necturus, 4%. These observations seem to indicate that the extent of the response of the animal to pituitrin is related to its natural habitat.

184. MODIFICATIONS IN THE THYROIDS OF THE ENGLISH SPARROW FOLLOWING INJECTION OF THYREOTROPIC HORMONE. Dorothea Starbuck Miller, University of Iowa. (Introduced by Paul L. Risley.)

Adult male and female sparrows were injected with a thyreotropic pituitary fraction extracted by the author according to the method of Greep. This preparation was found to have no stimulating action upon the gonads, but stimulated the thyroids. Each bird received a daily injection of 0.33 gm. equivalents of dry powder. Birds were killed at intervals to study the changes occurring in the thyroids. The volume of the glands was increased four to eight times over the normal size. Hyperplasia characteristic of the activated thyroid was observed microscopically. Twelve hours after the first injection, the height of the follicular epithelium is doubled. The amount of colloid in the vesicles is reduced, while a greater proportion of the colloid stains blue with Mallory's triple stain. Around the periphery of the blue-staining colloid mass are conspicuous chromophobe 'resorption vacuoles.' After 24 hours, the epithelial height is nearly three times normal. After 2 days of injections, colloid is absent from all the vesicles. The epithelial height is maximal, five to seven times normal. A few mitoses are found in the epithelium. After the third day, mitotic figures are fairly numerous. The follicles retain their normal rounded shape, with the tall epithelium filling the lumen. The interfollicular capillaries are congested with blood cells.

There is little further change in the histological condition through 12 days of injection. Recovery is rapid following the cessation of injections. Within a few days, the vesicles are filled with colloid and the epithelium has regressed to its normal height.

185. CHANGES IN BLOOD FATS FOLLOWING BILATERAL ADRENALECTOMY. E. H. Yeakel, Bryn Mawr College and Biological Laboratory, Cold Spring Harbor. (Introduced by E. W. Blanchard.)

Work previously reported from this laboratory upon the cholesterol partition between cells and plasma in normal and adrenalectomized suggests that some relation exists between adrenal cortical function and blood lipid regulation. In the present experiment whole blood and serum from normal, unilaterally operated, and bilaterally adrenalectomized cats was tested for total fatty acid and for phospholipid. Boyd's modification of Bloor's oxidative method was used. The results showed that normally the phospholipid and fatty acid content of the whole blood was a little greater than that of the plasma, but that following the extirpation of both glands, there appeared a marked change in the proportion of fatty acid and phospholipid in the cells and plasma. The cells showed a marked increase in lipid content in relation to the amount present in the plasma. It is suggested that in the absence of the cortical hormone, there occurs a shift of lipids from plasma to cells, or else a retention of these compounds in the cells as contrasted with a loss from the plasma. In either case, the change in lipid proportion in plasma and cells may be related to the property of cell membrane permeability.

186. THE EFFECT OF THYROIDECTOMY UPON LACTATION IN THE RAT AND GUINEA PIG.

W. O. Nelson and C. E. Tobin, Wayne and Yale Universities.

In a series of female rats which had been thyro-parathyroidectomized on the thirteenth to fifteenth days of pregnancy, twelve survived through gestation. These animals bore normal litters and in every instance raised their young. Routine weighing of each litter showed that the young of these thyro-parathyroidectomized mothers compared favorably in their growth rate with the litters of normal mothers. Four pregnant guinea pigs were thyroidectomized during the last week of pregnancy. In each instance the females delivered and raised normal litters.

It has been shown in earlier work that guinea pigs whose mammary glands had been developed by means of ovarian grafts or injections of theelin would lactate when the grafts were removed or the injections discontinued. This is true when the hypophysis is present. Two male guinea pigs that had carried functional ovarian grafts, and three males and one female that had received 40 R.U. of theelin daily for 28 days were thyroidectomized. At the time of operation the ovarian grafts were removed, or in the case of the injected animals, theelin was discontinued. In each case lactation was initiated and continued in amounts and duration comparable to animals whose thyroids had not been removed.

This study is of interest in that it demonstrates that the thyroid gland, unlike the adrenals, may be removed without seriously interfering with lactation. It is not claimed that thyroidectomy or thyro-parathyroidectomy does not affect lactation in any way, but it is apparent that lactation in animals deprived of these glands is sufficient to support normal growth of young animals.

187. THE EFFECT OF VARIOUS SEX HORMONES ON THE TESTES OF HYPOPHYSECTOMIZED RATS.

W. O. Nelson, Wayne and Yale Universities.

It was recently shown (Nelson and Gallagher, '36) that extracts of male hormone prepared from urine would prevent the degeneration of the spermatogenic tubules that usually follows hypophysectomy, if treatment was begun within a few days after operation.

These experiments have been confirmed with testosterone and androsterone, and are being extended to include dehydroandrosterone and other synthetic androgenic substances.

The injection of as little as 1 rabbit unit (Corner) of progestin daily for 22 to 25 days after hypophysectomy has been as effective as male hormone in preventing degeneration of the spermatogenic tubules. One-half rabbit unit daily had only a slight effect. In contrast to the marked stimulation of the sex-accessory organs obtained with the male hormones, the sex-accessories of the progestin-treated animals were similar to those of untreated hypophysectomized controls. Experiments with crystalline progesterone are in progress.

A series of hypophysectomized males have received from 30 to 400 R.U. (200 to 2800 I.U.) of oestrone daily for 18 to 22 days after operation. In no instance was there any evidence that oestrone would prevent testicular atrophy. Indeed, the testes of these animals were regularly smaller than those of untreated hypophysectomized controls.

Although progesterone and the androgenic hormones are closely related chemically, the absence of stimulation of the sex-accessories in the progestin-treated animals suggests that the factor responsible for the stimulation of spermatogenesis may not be identical with the factor concerned in the stimulation of the accessories.

188. THE EFFECT OF OESTRONE AND OF PITUITARY EXTRACTS ON THE MAMMARY GLANDS OF THE RAT. W. O. Nelson and C. E. Tobin, Wayne and Yale Universities.

In a large series of young adult normal and spayed female rats it was shown that the daily administration of oestrone (30 to 100 R.U.) would result in marked proliferation of the mammary ducts and alveoli. The stimulation was somewhat more pronounced in normal animals since the treatment with oestrone prolongs the life of the corpora lutea and results in the combined action of oestrin and progestin. However, the results in spayed animals are so conclusive that there can be no doubt of the activity of oestrone alone in stimulating alveolar proliferation in the rat.

Two groups of thyroidectomized female rats were injected with oestrone. In one group the ovaries were also removed. The effect of oestrone on the mammary glands of these two groups was identical with that observed in animals with their thyroids intact.

In hypophysectomized rats, with or without their ovaries, oestrone has very little effect on the mammary glands. The treatment of such animals with purified lactogenic hormone preparations in addition to oestrone has been no more effective than oestrone alone. However, animals treated with both oestrone and a crude pituitary extract have shown well-developed mammary glands. This finding indicates that the failure of the mammary glands of hypophysectomized rats to respond to oestrone is probably not due to the absence of the lactogenic hormone, but rather to some other factor or combination of factors.

189. INDICATIONS OF A CERTAIN CONSTANCY IN THE CHARACTER OF HEAT IN INDIVIDUAL GUINEA PIGS. William C. Young, Edward W. Dempsey, John L. Boling and Carl W. Hagquist, Brown University.

An earlier study directed attention to the regularity with which heat of a certain length and character recurred in individual animals for as many as five consecutive heat periods. In some animals it was of long duration, 10 to 16 hours; in others its length varied between 6 and 9 hours; in a third group it was of less than 5 hours duration or missed entirely (J. Comp. Psychol, 1935). Recently data have accumulated from twenty animals observed continuously, day and night from four to twelve consecutive heat periods during 1935–1936 and four consecutive heat periods a year later.

The records of each of five animals whose behavior was uniformly extreme in one way or another were nearly identical for the two periods of observation. The behavior of twelve animals for the two periods was strikingly similar. For only three animals were the behavior records for the two periods conspicuously different.

Because some selection in the choice of animals was unavoidable, it is doubted that such striking regularity would be found in as large a percentage of animals from the average colony. Nevertheless, considerable constancy of individual differences is very common.

Interpretation depends partly upon a study of the ovarian condition now in progress. Material which has thus far become available, however, suggests, in addition to ovarian factors, the existence of extra-ovarian control or regulation.

190. THE MEASUREMENT OF SEX DRIVE IN THE FEMALE GUINEA PIG. William C. Young, Edward W. Dempsey, Carl W. Hagquist and John L. Boling, Brown University.

A common characteristic of such generally employed methods of measuring sex drive in laboratory rodents as activity cages and obstruction devices is a dependence upon the activity of the animal whose sex drive is to be measured. In the normal guinea pig, as well as the spayed individual in which replacement therapy is attempted and the animal subjected to operative treatment of the ovaries, a behavior frequently occurs which would do much to invalidate determinations obtained by the methods referred to above. This is the mounting activity about the time sexual receptivity begins. Undoubtedly mounting is associated with heat, but the exact relationship is in need of elucidation.

Were this behavior indicative of heat, or were its frequency indicative of the vigor or length of heat, no confusion would result. We have found, however, that although mounting, when it occurs, appears to account for much of the activity displayed during heat, it is not a measure of temporal or intensity differences in heat. A periodically or continuously active animal may not be sexually receptive once during the period of observation while a relatively quiet animal may experience long, regularly recurring periods of heat and give vigorous behavioral responses when stimulated.

It is suggested, therefore, that in the guinea pig, determinations of the length of heat accompanied by estimates of the intensity of the response in terms of lordosis and vulvar dilatation provide the most accurate means of measuring sex drive.

191. HOMOTRANSPLANTATION OF PREPUBERTAL RAT TESTES TO THE ANTERIOR CHAMBER OF THE EYE. Clarence D. Turner, University of Missouri and University of Georgia. (Introduced by Mary J. Guthrie.)

Testes from newborn animals were transplanted bilaterally to the eyes of normal postpubertal males and females and to castrate adults of both sexes. A total of 118 such transplants have been made, and 94% of them have become functionally incorporated. These were allowed to remain in the eyes for periods ranging from 10 days to 10½ months. Calcification and fatty infiltration of the grafts have not been observed in any case. Connective tissue invasion was exceedingly infrequent and was never extensive. In all types of hosts spermatogenesis was completed in tubules near the cornea but was most extensive in castrates. Grafts persisting for 10 months in males bilaterally castrated for the same period contained spermatozoa in approximately one-fifth of the tubules. Fully differentiated spermatozoa could be identified in fixed preparations and observed in teased supravital material. The transplants have been found to maintain all of the male accessories in a secretory condition for periods up to 10 months. Castration changes in the anterior pituitary gland were retarded.

192. THE SEXUAL BEHAVIOR AND SECONDARY SEXUAL CHARACTERS OF GONADECTOMIZED FISH. G. K. Noble and K. F. Kumpf, American Museum of Natural History.

Male Hemichromis bimaculatus will exhibit typical movements of courtship, fertilization and brooding for 202 days after castration. During this period a male may enter into as many as thirteen successive spawnings with a normal female. Males castrated before their first spawnings may take part in at least eleven successive spawnings during a period of 146 days. Castrated males develop nuptial colors and genital tubes at each spawning. In striking contrast gonadectomized female Hemichromis placed with ripe males fail to exhibit any sex activity although the behavior patterns of this sex resemble those of the male closely.

The breeding cycles of Hemichromis bimaculatus are initiated by the female. Castrated males may be induced by introducing ripe females to shorten their intervals between sex activity to as little as 1 or 2 days. The cycles of the normal female may be shortened by implants of frog pituitaries.

Betta splendens resembles Hemichromis in that gonadectomized males unlike gonadectomized females will engage in typical sex activity with normal individuals of the opposite sex. Spayed Xiphophorus helleri unlike spayed Betta are sexually attractive to males of their own species. This is correlated with the different roles played by the female in the courtship of these forms.

Normal breeding behavior may be induced in spayed Betta by ovarian extracts, while most of this behavior may be restored to spayed Hemichromis by the same injections. Frog pituitaries induce the growth of genital tubes in spayed Hemichromis but restore only the first stages of courtship.

Cytology

193. ULTRACENTRIFUGING AND THE FIRST CLEAVAGE PLANE IN ASCARIS SUUM. H. W. Beams and R. L. King, State University of Iowa.

The components of the protoplasm of fertilized uncleaved eggs of Ascaris suum stratify upon ultracentrifuging into three layers: a) yolk (mitochondria?) centrifugally, b) clear, apparently homogenous, protoplasm equatorially, and c) fat centripetally. These become redistributed within 12 hours after removal from the centrifuge, and the eggs eventually cleave and embryonate normally. In some cases the fat is completely separated from the rest of the egg, which also develops normally with the fat remaining isolated. Eggs will cleave while in the centrifuge at 150,000 × gravity. Here the mitotic spindle is oriented so that the first cleavage plane passes perpendicular to the 'yolk' pole and the 'fat' pole, through the protoplasmic layer, but does not usually penetrate the massed materials at either pole. The first cleavage may result in two, three or four cells, only two of which contain nuclei in any case. When two cells result, each contains a nucleus and half the protoplasm, one includes all the fat, the other all the yolk. When four cells result, we have a 'yolk cell' without nucleus, a 'fat cell' without nucleus and two cells, each with half the protoplasm and a nucleus. When three cells result either the fat or the yolk has been included in one of the nucleated cells, and either the yolk or the fat form a separate 'cell.' Most of the embryos resulting from such eggs are highly abnormal. It is thought that the fundamental polarity of the egg cell has been disturbed.

194. THE CHROMOSOMES AND CHROMATOID BODIES OF THE WESTERN CRAYFISH, POTAMOBIUS TROWBRIDGEI STIMPSON. Nathan Fasten, Oregon State College.

These chromosome studies were made on testicular cells of the western crayfish, Potamobius trowbridgei Stimpson, a species abundant in many of the fresh water streams of Oregon. Both sectioned material and smear preparations were available for study. The diploid count in polar views of spermatogonia revealed 184 compact, spherical chromosomes, distributed throughout the equatorial plane of the spindle. The haploid number, after reduction, showed ninety-two spherical chromosomes, about twice as large as the spermatogonial chromosomes, and distributed similarly throughout the equatorial plane of the spindle.

During the growth stages of the primary spermatocyte, two chromatoid bodies make their appearance within the cytoplasm, one slightly larger than the other. These stain almost as heavily as the chromatin, but are surrounded by clear areas. During the reduction and equation divisions, the two chromatoid bodies may behave in a manner to produce four types of spermatids: 1) with the two chromatoid bodies, 2) with the single large chromatoid body, 3) with the single small chromatoid body, and 4) with no chromatoid bodies. The fate of these chromatoid bodies has not, as yet, been determined.

195. THE CENTRIOLE IN THE PARTHENOGENETIC AND FERTILIZED EGGS OF HABROBRACON JUGLANDIS. Elias M. Spotkov, Washington Square College, New York University. (Introduced by Alfred F. Huettner.)

Cyto-centrosomes containing centrioles but lacking asters originate de novo in great number during the early maturation period in fertilized and unfertilized eggs of Habrobracon. The evidence from the eggs favors the origin of the cyto-centrosomes from the germinal vesicle by the extrusion of granules and are achromatic, reacting negatively to the Feulgen nuclear test.

Asters similar to those in artificially parthenogenetic echinoderm eggs and enucleated fragments of other forms make their appearance around many of the cyto-centrosomes, thus forming cytasters. These may number from thirty to fifty in an egg and are manifest only after the female pronucleus has established an association with a cyto-centrosome, which thus becomes the nuclear centrosome.

In the parthenogenetic egg the centriole of the nuclear centrosome is continuous from one cell generation to the next, while the centrioles of the cyto-centrosomes, unassociated with nuclear material, are not perpetuated and disappear by the fifth cleavage. In the fertilized egg, however, the origin of the nuclear or first cleavage centrioles may be twofold: a) Maternal—from two cyto-centrosomes, one becoming associated with the female pronucleus, and the other with the male pronucleus. Each centrosome becomes located at a pole of the first cleavage spindle. b) Maternal-paternal—from two centrosomes, one the sperm centrosome which becomes located at one pole of the first mitotic spindle, the other a cyto-centrosome connected with the female pronucleus and located at the opposite pole.

196. The effect of anterior pituitary extract on the formation of meiotic spindles in oocytes. Katharine R. Jeffers and Mary J. Guthrie, University of Missouri.

Following a suggestion (Pincus, '36) that the formation of the first polar body in the oocyte may be conditioned by an increase of pituitary hormones in the blood, oocytes of atretic follicles in normal bats and those injected with anterior pituitary extract were examined for spindles, and counted. In bats atresia occurs before antrum formation, but only oocytes that were mature with respect to cytosomal differentiation have been considered. Five bats given three injections in January and killed 5 to 10 days after the final injection, and five bats similarly treated and killed in February were examined. Ovulation had occurred in all except one animal in which the luteinized follicle contained an oocyte with a spindle. The average number of atretic follicles is less in injected than in control bats. In the injected individuals 18.2% of the 148 oocytes contained spindles; in ten controls killed at comparable periods spindles occurred in 27.4% of the 190 degenerating oocytes. In five additional animals killed 1 to 2 days after the final injection, 7.7% of the oocytes in seventy-eight atretic follicles had spindles. These results indicate that pituitary extract which induced ovulation had an inhibiting effect on the formation of spindles in mature oocytes in atretic follicles.

197. The relationship of the Golgi material to secretion in the basophilic cells of the hypophysis in the albino rat. Arthur J. Gatz, Carleton College. (Introduced by D. E. Minnich.)

The Golgi material in the basophilic cells of the anterior lobe of the hypophysis is not static. The material undergoes modifications in size and morphology. It has a definite relation to the secretory cycle of the basophil in which the following steps may be outlined: a) The presence of osmiophilic granules within the central portion of the Golgi material. b) The presence of droplets surrounded by an osmiophilic girdle within the central portion of the Golgi material. c) The presence of droplets in the osmiophilic periphery of the Golgi material. d) The presence of secretion droplets surrounded by an osmiophilic girdle at the periphery of the small forming vacuoles, as well as at the periphery of the large vacuoles in the vacuolated cells. e) The presence of osmiophilic granules and thread-like particles located in the cytosome of the large vacuolated cells. These fragments are apparently the remnants of the osmiophilic girdle which had surrounded the secretion droplet. In my opinion, the osmiophilic girdle, derived from the Golgi material, serves an enzymatic function or acts as an intracellular center of chemical synthesis. If the Golgi material serves an enzymatic function it apparently must be used up during the process. If such were the case the elimination of the Golgi material as a distinct body may be explained. Doubtless some of the material is used up, while the remainder forms the discrete granular and thread-like particles of osmiophilic substance which have been observed in the cytosome.

Morphology

198. TWO TYPES OF RESPIRATION IN ONCHIDIUM. Leslie B. Arey, Northwestern University Medical School.

Onchidium is a pulmonate mollusc which has adapted itself to marine littoral life. The presence of lungs, long denied, has been satisfactorily established but the relative use of pulmonary and cutaneous respiration has not been entirely clear.

Onchidium floridanum, an Antillean species common at Bermuda, respires cutaneously, and chiefly through its numerous though short mantle papillae, both when submerged and when quiescent in the air. Under both of these environmental conditions the pulmonary aperture remains closed and the lungs are inoperative. When given a choice, most animals select the air for long periods of quiescence.

When an animal, out of water, is actively crawling, the posterior portion of the mantle is raised from the substrate to expose the opened pulmonary orifice and facilitate pulmonary respiration, the existence of which is demonstrable. The lungs could be of considerable service during such periods of activity and it may be that they actually dominate the terrestrial respiration of active animals. Such a conclusion, however, does not rest on any sound evidential basis and the lungs may serve merely as accessory respiratory organs when the animals are out of water and active.

If the lungs be subordinate in the terrestial respiration of active Onchidia, in addition to their disuse during aquatic life and inactive air existence, then the lungs of this pulmonate (which has secondarily acquired marine habits) have indeed sunken to a low functional state.

199. PLACOID SCALE TYPES AND THEIR DISTRIBUTION IN SQUALUS ACANTHIAS. Leonard P. Sayles, College of the City of New York and Marine Biological Laboratory.

In 55 to 62 cm. dogfish about ten different types of placoid scales may be distinguished, mainly on the basis of differences in the shapes and sizes of the longitudinal and transverse spine components. The trunk and tail regions have scales shaped like the placoid scales ordinarily depicted. Scales of the dorsal side have both components broader and shorter than do ventral ones. Laterally there is a transition between these types. In addition, tricuspid scales are fairly numerous dorsally and laterally. The tip of the snout is covered with large, flattened scales which radiate from a point slightly dorsal on the anterior tip. On the head are many tricuspid scales with secondary cusps set more posteriorly than on trunk tricuspids.

A number of relatively small scaleless areas occur. These are usually bordered by marginal scales with large, broad transverse components and reduced longitudinal ones. Somewhat similar scales are found along the edges of fins but small scales of the dorsal body type predominate over most of the fin.

Several rows of comb-like scales are found in the regions of each gill septum adjacent to the gill filaments, both externally and internally. Stomodeal denticles are not found on the roof of the mouth and pharynx except in so far as the dorsal margins of the internal gill slits contribute to this region. Such denticles are also found in the covering of gill arches and of the floor of the pharynx from the first gill slit to esophagus.

200. THE PROBLEM OF SPECFICITY OF FORM IN CUTICULAR SCALES, AND OF SPECIFICITY IN HAIR-SHAFT STRUCTURE ASSOCIATIONS AMONG THE MAMMALIA. Leon Augustus Hausman, New Jersey College for Women.

In a study of the form and structure of the cuticular scales occurring midway between the base and the tip of hair shafts from the mid-dorsal region of 300 species of mammals (exclusive of Chiroptera) undoubtedly specificity of scale structure and form could not be established. Here (as previously by Hausman Wynkoop, Smith) the morphological scale groups were found regularly correlated with the diameters of the hair shafts on which they occurred. Minute variations of the free distal edges of the scales were frequent, but not correlated with species nor larger taxonomic groups. However, the intermediate bat (Mormoops intermedia) possesses a scale form not yet found in any other mammal; and in the Javan pigmy bat (Tylonycteris pachypus) a scale type was encountered transitional in form (and hence new) between the coronal type of scale (of the Chiroptera), and the more or less imbricate type, or soricid type (of the Soricidae). A study of a large series of hairs of Chiroptera from the American Museum of Natural History and the Smithsonian Institution is now being carried on. At present it may be said that specific microscopic structural characters of hair shafts of mammals may not be found in any one structural element, but in the various associations of structure, form, relative sizes of, 1) cuticular scales, 2) medullae, 3) pigment granules (together with their patterns), 4) fusi, or 'air vesicles,' and of the status of, 5) the cortex and its diffuse pigment.

201. THE OCCURRENCE OF UNILATERAL AND BILATERAL OVARIES IN RAPTORIAL BIRDS. L. V. Domm, The University of Chicago.

It has been assumed that, the occurrence of unilateral ovaries is general in birds. However, in reviewing the literature, one comes to the conclusion that bilateral ovaries may be somewhat more common among the Raptores. Since this condition is rare in the birds we have studied we became interested in determining its frequency and distribution within this order. We have examined individuals from two groups namely owls and hawks.

Dissection of adult female owls representing five species reveals unilateral development of the ovary and oviduct. In only two instances did we find cortical tissue macroscopically on the right side. No oviduct was found on the right. The condition of the urinogenital system is quite similar to that found in the domestic fowl.

Dissection of adult female hawks belonging to seven species reveals bilateral development of ovaries in varying degrees. It appears that in some species right ovaries of varying sizes are invariably present and that in at least one species (marsh hawk) they are almost invariably as large as the left ovary and in some instances larger. In the sparrow hawks examined the right ovary was always considerably smaller than the left and in two of these no trace of right ovary was evident macroscopically. Several red-tail hawks with no trace of right ovaries were also encountered.

That ovulation occurs also in the right ovary was shown by a number of individuals taken during the breeding season. The left oviduct therefore must accomodate both ovaries since no right oviducts were found.

202. WEIGHTS AND LINEAR MEASUREMENTS OF THE DIGESTIVE SYSTEM OF THE ADULT CAT. Homer B. Latimer, University of Kansas.

Since the publication of the data on the prenatal growth of the digestive system of the cat, a series of 104 adult cats (fifty-two male and fifty-two female) has been studied. The following seven parts were weighed: entire digestive tube, esophagus, stomach, small intestine, large intestine, liver and pancreas. The lengths measured were: entire tube, esophagus, small intestine and large intestine.

Most variable are the weights of the liver and pancreas in the males. All of the parts are significantly greater in the males except the weights of the large intestine and pancreas and the lengths of the esophagus and large intestine.

In general, the weights of the parts of the digestive system are better correlated with body weight than are the linear dimensions correlated with body length. The lengths of the large intestine form positive, though non-significant, correlations with both body weight and body length. The linear dimensions of the esophagus and the large intestine correlated with the length of the entire tube are not significant and are lower in the males.

A comparison of the percentage weights in the early fetus, at birth and in the adult shows that the digestive tube increases to its maximum at birth and then decreases slightly. The liver decreases from its early maximum and the pancreas increases in the fetal stage and very slightly thereafter. The relative proportions (both linear and ponderal) of the parts of the digestive system change but little after birth.

203. A QUANTITATIVE STUDY OF THE SKELETON OF THE MOURNING DOVE, ZENAIDURA MACRURA CAROLINENSIS. Homer B. Latimer and C. Willet Asling, University of Kansas.

Forty carefully prepared skeletons, borrowed from the Natural History Museum, were used in this study. The skull and six long bones were weighed. Twelve dimensions of the skull and the lengths of the same long bones were measured. All paired bones were weighed and measured separately and the sum of the weights and the average of the lengths were used in the first computations.

The humerus is the least variable in weight (coefficient of variation, 5.91) and in length. The femur is nearly three times as variable in weight. The skull weight has a coefficient of 9.42.

The lengths of the long bones are less variable than the dimensions of the skull. The average of the first is 2.77 and of the skull dimensions, 5.14. The bitemporal diameter is the most constant of the skull dimensions and the interorbital diameter the most variable. The skull is more variable in length than in height and both of these dimensions are more variable than the bitemporal width.

There is a rather marked symmetry in the lengths of the paired bones and probably also in the weights. The femur and ulna are heavier in over 50% of the cases on the left side and the tibiofibula on the right side. In no other case is there a percentage of 50%.

Protozoölogy

204. THE INFLUENCE OF CERTAIN AUXINS ON GROWTH IN PROTOZOA. Alfred M. Elliott, Minnesota State Teachers College.

Certain plant hormones, 3-indole acetic acid (heteroauxin), B-3-indole propionic acid, and gamma-3-indole butyric acid, were tested for their effect as growth stimulants on three species of protozoa in pure cultures. Comparative studies were made with a chlorophyll-bearing flagellate, Euglena gracilis, a colorless euglenoid flagellate, Astasia sp. and a holotrichous ciliate, Colpidium striatum. The experiments were conducted under similar conditions and the compounds were tested over a wide pH range (3.5 to 8.8 at 0.5 pH intervals). Growth in the case of Euglena was more than doubled in media containing gamma-3-indole butyric acid (concentration 1: 10,000); slight acceleration was observed with the other two indole compounds. In the case of Astasia and Colpidium acceleration was negligible with all three compounds and in most instances the substances were toxic. Results seem to indicate that the auxins tested stimulate growth when the cell contains chlorophyll but are without effect on colorless protozoa.

205. THE RELATION BETWEEN THE FOOD SUPPLY AND THE RATE OF LOCOMOTION IN THE MARINE AMOEBA, FLABELLULA MIRA SCHAEFFER. D. L. Hopkins, Duke University.

The amoebae were fed on pure strains of bacteria. Six different kinds of bacteria were used, each of which alone afforded sufficient nutriment for normal growth and reproduction of the amoeba. The bacteria were grown in a dextrose-peptone solution, and then washed thoroughly by centrifuging through three changes of sterile distilled water. The amoebae were then inoculated into various concentrations of each of the six kinds of bacteria in artificial sea water. The amoebae were allowed to feed, grow and reproduce for 5 days. At the end of this period amoebae were removed from each of the concentrations of bacteria, placed in fresh artificial sea water at 21.6°C. and the average rate of locomotion observed. It was found within the limits of the experimental error involved in measuring the rate of locomotion, that regardless of the concentration or kind of bacteria which had been available as food the rate of locomotion was the same.

The rate of growth and reproduction was also roughly measured and was found to increase as the concentration of food increased, except in the case of the highest concentration of bacteria used, (one billion or more per cubic centimeter) there was a slight decrease in the rate of growth and reproduction.

206. THE REMOVAL OF TOXIC SUBSTANCES FROM FRESHLY PREPARED ARTIFICIAL SEA WATER BY MEANS OF SODIUM SILICATE. D. L. Hopkins, Duke University.

Artificial sea water when freshly made up with ordinary c.p. chemicals shows a slight toxic effect upon amoebae. If the artificial sea water is allowed to stand for a few weeks in a clean ordinary glass bottle the ability to produce the toxic effect is lost. Presumably the very small traces of toxic heavy metal ions are removed from the solution by adsorption onto the surface of the glass. It has been found that the addition of very small traces of Na_2SiO_3 to fresh artificial sea water will within 10 hours precipitate from the sea water a sufficient amount of the toxic substances, so that the sea water is no longer toxic to the amoebae. Artificial sea water thus treated with Na_2SiO_3 will support continuous growth and reproduction much better if a trace of iron is added to replace that removed by the Na_2SiO_3.

207. The nature and significance of the Golgi material in the marine amoeba, Flabellula mira Schaeffer. D. L. Hopkins, Duke University.

The Golgi material in this amoeba in the normal living condition is uniformly distributed throughout the cell. It may be caused to aggregate into distinct bodies by treatment with the vital dye neutral red. The usual methods for bringing out the Golgi material, also cause an aggregation of the material into distinct bodies. Treatment with isotonic NH_4Cl causes the aggregation of this material into very small granules. Whether the Golgi material is uniformly distributed throughout the cell, or aggregated into comparatively large bodies by neutral red, or into very small granules by NH_4Cl, heat destroys the ability of the material to reduce osmium tetroxide. Identically the same temperature conditions destroy permanently the locomotor mechanism of the amoeba.

When the amoebae are adapted to a temperature between 15° and 20°C. heating suddenly at 43°C. for 4 hours or to 50 for 15 minutes will destroy permanently the locomotor mechanism and the ability to reduce osmium tetroxide. A sudden increase to 43°C. for 30 minutes or a gradual increase to 50°C. will not destroy the locomotor mechanism permanently and the amoebae will still reduce osmium tetroxide.

208. Growth of Euglena stellata in darkness. R. P. Hall, New York University.

Euglena stellata, according to Dusi ('33), is an 'obligate phototroph' dependent for growth upon exposure to light. With a peptone-sodium acetate medium at pH 7.0 the writer has obtained growth of E. stellata in darkness through four successive transfers over a period of 2 months. The first series was inoculated from a stock culture grown in light. The second, third and fourth transfers were inoculated from a tube of the first, second and third series, respectively. Since inoculation of each of the last three transfers involved a 1: 19 dilution of the inoculum, and the final concentration of flagellates in the fourth series was greater than the initial concentration in the first, the actual increase in population through the four transfers was rather large.

In several species of Euglena the writer has observed that sodium acetate partially inhibits multiplication at a pH of 6.0 or lower. Hence, Dusi's conclusion that E. stellata is an obligate phototroph may possibly be based upon his use of sodium acetate in a medium of unfavorable pH.

209. Effects of $MnCl_2$ on growth of certain protozoa. R. P. Hall, New York University.

Manganese chloride, in molar concentrations ranging from 10^{-9} to 10^{-4} decreased the growth of Astasia sp. and Colpidium campylum in peptone media. With Euglena anabaena, grown in light in similar peptone media known to satisfy minimal requirements for growth, accelerating effects were observed over a concentration range of 10^{-9} to 10^{-5} molar. Acceleration of growth in light in a solution of inorganic salts was much greater than that observed in the peptone media. When the flagellates were grown in darkness in peptone media, the acceleration was usually slight and was apparent only in the lower concentrations of $MnCl_2$. Preliminary observations indicate that the apparent accelerating effect of this salt on growth of Euglena may bear some relation to pH of the medium.

210. Growth of two species of Astasia in relation to pH of the medium. H. W. Schoenborn, New York University. (Introduced by R. P. Hall.)

Optimal pH for growth in various types of media has been determined for Astasia sp. and Astasia ocellata (?) in bacteria-free cultures. Both strains were isolated by Dr. T. L. Jahn. Euglena gracilis (Pringsheim strain), also in bacteria-free cultures, was used for comparison, since the optimal pH of this species had already been determined by other workers. In a salt medium containing Difco tryptone (0.2%), the optimal pH for Astasia sp. and E. gracilis was below 7.0, being near 6.0 for the former and approximately 6.4 for the latter. Astasia ocellata, on the other hand, showed an optimum above 7.0, and usually near pH 7.5. With the addition of sodium acetate (0.15%) to this medium, the optimal pH for each species was shifted toward the neutral point.

211. The effects of magnesium and calcium on growth in Chilomonas paramecium. S. O. Mast and D. M. Pace, Johns Hopkins University.

Chilomonas grows well in water containing $NaC_2H_3O_2$, NH_4Cl, K_2HPO_4 and $MgSo_4$. Na and Cl are not necessary. This indicates that Chilomonas requires only eight elements.

If magnesium is omitted or if its concentration is very low (0.000001 M or less) the chilomonads grow but they do not divide. This results in the production of monsters. Some of these have several flagella and nuclei, but they contain a normal amount of starch and fat. If magnesium is added in proper concentration the monsters divide often and produce normal specimens. Elimination of magnesium, therefore, prevents division of the cell but it does not prevent division of the nucleus and synthesis of starch and fat.

If magnesium is in optimum concentration (0.0013 M) the division rate at 24°C. is 3.325 per day. If calcium in optimum concentration (0.0001 M) is added in place of magnesium the division rate is 3.7 per day. If magnesium and calcium are added in optimum concentrations it is 3.9 per day. Calcium can, consequently, be substituted for magnesium.

Elimination of calcium has the same effect as elimination of magnesium.

212. THE EFFECT OF ULTRACENTRIFUGING ON PARAMECIUM, WITH SPECIAL REFERENCE TO RECOVERY AND MACRONUCLEAR REORGANIZATION. R. L. King and H. W. Beams, State University of Iowa.

Paramecium caudatum becomes much elongated upon centrifuging at 21,000 times gravity. The chromatin is sometimes forced from the achromatic matrix of the macronucleus. The materials in the cell are redistributed according to their relative specific gravities as follows: At the centrifugal end of the cell, crystals, layer of fluid, micronucleus and macronuclear chromatin, food vacuoles and neutral red inclusions, achromatic matrix of the macronucleus, endoplasm with large clear alveoli, and fat, at the centripetal end of the cell. The contracting vacuole is displaced sometimes but not the feeding canals or pore. The membrane of the contracting vacuole is temporary. In some cases the crystals, micronucleus and macronuclear chromatin may be extruded. Animals which survive centrifuging regain their usual shape and the disturbed materials return to the usual distribution rapidly. Some times the crystals remain in large compact masses and are so passed to the daughter cells upon fission. The two components of the macronucleus do not fuse, the macronuclear chromatin regenerates an achromatic matrix, and division is somewhat delayed. The old macronuclear matrix persists over a long time and sometimes interferes with division. Animals which have no micronucleus may survive and divide, but no amicronucleate races have been established. Apparently macronuclear chromatin is necessary for the survival and division of P. caudatum; in the absense of the macronuclear chromatin is necessary for the survival and division of P. caudatum; in the absence of the macronuclear chromatin no replacement occurs from the micronucleus.

213. CYTOLOGICAL NOTES ON THE CHLAMYDODONTIDAE. Mary Stuart MacDougall, Agnes Scott College.

In cytological studies of the genus Chilodonella from 1925 to 1936, attention has been called to the fact that at the prophase of the first maturation division material has been eliminated. This substance has been designated as plastin, debris, a mass, etc. Recently, by use of Fuelgen and other stains, it has been indicated that this material is chromatin. In studies of the conjugation of eight species of this genus, this elimination of chromatin from the micronucleus always takes place at the time of the formation of the chromosomes, often obscuring the latter. As has been reported, the elimination of chromatin by the macronucleus at the time of division varies greatly in the three series of Chilodonella.

The dividing macronucleus of Chalmydodon Sp. shows elimination of chromatin in a manner quite different from the situation in the genus Chilodonella. This nucleus has a 'kernspalt' and lacks the endosome of the typical Chilodonella macronucleus. Several forms having a macronucleus with a 'kernspalt' are included in the genus Chilodonella. Material has not been available for study of these forms. It is more than probable that they should not be included in this genus, a matter to be determined as soon as suitable material is available for study.

214. The vacuolar system of the Euglenida. Libbie H. Hyman, American Museum of Natural History.

Observations on living Euglena, Phacus, Peranema and Entosiphon show that the usual account of the vacuolar system of the euglenoid flagellates is incorrect. There are no accessory vacuoles opening into a main vacuole. The contractile vacuole is impermanent; it discharges into the gullet base ('reservoir' of Klebs) and disappears without trace, having no connection with the next vacuole. As each contractile vacuole reaches diastole, there appear near it a few to several small vacuoles which after it has discharged flow together to form the next vacuole. The latter is therefore large at its first appearance and does not increase much thereafter. The system therefore differs from that of other Protozoa only in that the small vacuoles whose union produces the vacuole appear before the latter discharges rather than after. This circumstance has given rise to the mistaken idea that the small vacuoles are accessory vacuoles.

215. A study of Euglena rubra (Hardy, '11). Leland P. Johnson, Iowa Lakeside Laboratory and State University of Iowa. (Introduced by R. L. King.)

Great numbers of Euglena rubra, Hardy, '11, appeared in Lake West Okoboji, during the summer of 1936. This species has an elongated body, is rounded anteriorly and protrudes posteriorly into a colorless rounded tip. It is 76 to 163 μ long and 30 to 36 μ wide. The flagellum is slightly longer than the body. This species contains large quantities of haematochrome and paramylum bodies, the latter making observations difficult. Chloroplasts appear spindle-shaped in the organism but assume a disc-shape when the pressure of paramylum bodies is removed. The haematochrome is evenly distributed in sunlight but is concentrated centrally when shaded; this makes the organism appear red in bright light and green in shade. Resting and reproductive cysts are spherical (34 to 47 μ in diameter). Division is similar to that described for other species except the gullet appears to divide or form a second before the reservoir divides. Upon ultracentrifuging three distinct layers appear; the paramylum concentrated at the centrifugal pole, the haematochrome at the centripetal pole, and the chloroplasts in a central position. The materials of these three layers return to their normal distribution rapidly; this is a great aid in the observation of chloroplasts.

The parasite, Spaerita dangeardi, was observed in Euglena ruhra. The earliest stage observed was 5 μ in diameter, the largert was 38 by 15 μ and possessed a nucleus 9 μ in diameter. The formation of zoospores (2 μ in diameter), was also observed in S. dangeardi.

216. A colorless euglenoid flagellate, Khawkinea halli, n. gen., n. sp. Theodore Louis Jahn and Wendell R. McKibben, State University of Iowa and Iowa Lakeside Laboratory.

A colorless euglenoid flagellate similar to Astasia ocellata Khawkine is described in detail. The type of flagellum insertion (bifurcated at base with flagellar swelling) indicates that it is more closely related to the genus Euglena than to the genus Astasia. However, for clarity of generic distinctions it is proposed to erect a new genus of the family Euglenidae to include the permanently colorless species of Euglena and the stigma-bearing species previously assigned to the genus Astasia. The new genus differs from the genus Euglena only in that the cells are permanently lacking in chloroplasts. The new genus is named Khawkinea, and the present species which differs from the Astasia ocellata of Khawkine in the length of the flagellum, is designated as Khawkinea halli.

Both binucleate and anucleate individuals were found in bacteria-free cultures in peptone and Na-acetate but not in bacterized hay infusions or in bacteria-free cultures in plain peptone medium. From observations of the violent metabolic movements which sometimes precede division in the acetate medium and from a study of stained preparations it was apparent that the binucleate cells were formed by abnormal division rather than by copulation.

217. LICNOPHORA LYNGBYCOLA, N. SP. E. Fauré-Fremiet, Collège de France.

A new species of Licnophora has been found at Woods Hole and at Concarneau, which is peculiar by its constant association with a Cyanophycea of the group of the Lynbyae, which it uses as a support. The attaching disc is adapted morphologically to this biological condition.

218. ON THE OCCURRENCE OF TILLINA IN MINNESOTA, WITH A DESCRIPTION OF SOME NEW MORPHOLOGICAL STRUCTURES. John P. Turner, University of Minnesota.

A species of the 'rare' holotrichous ciliate Tillina, resembling T. magna has been found in a Minnesota pond on 3 successive years, cultivated abundantly for 3 years on timothy hay, and studied for details of the life history and morphology.

The uniformly ciliated body is 200 μ long and 130 μ broad, reniform, with a caudal twist caused by the spiraling of the oral groove posteriorly from the mouth. The cytosome is large and hemispherical, is located mid-ventrally, and leads into a large and complex cystostomal chamber containing a screen-like membrane.

Fission into two or four daughters occurs in a spherical capsule which is larger than the protective cyst. Division of the four to fourteen micronuclei occurs anywhere in the life cycle. The mouth and undulating membrane become visible before emergence from the capsule. No conjugation has been observed.

In the protective cysts a short rod-shaped macronucleus and several micronuclei are visible as in all other stages.

In active animals, blackish pigment rods are usually (not always) present and are generally (not invariably) concentrated in the posterior lobe of the twisted body.

The single posterior contractile vacuole is fed by seven to nine definite canals arranged fan-wise, each originating in an ampulla near the anterior margin of the body. The ampullae collect watery vacuoles and pass the contents back through the canals to the contractile vacuole.

Ecology

219. TEMPERATURE CHANGES IN THE OKOBOJI LAKES, SUMMER, 1936. Theodore Louis Jahn and Aubrey Bryant Taylor, Iowa Lakeside Laboratory and State University of Iowa.

As a type of aquatic environment Lake West Okoboji is particularly interesting in that the surface and bottom temperatures differ widely from year to year and in that they may or may not differ considerably from each other. In spite of a depth of 40 meters the development of a thermocline seems to be entirely dependent upon the wind-sunshine balance in any particular summer.

During the summer of 1936 numerous measurements were made with a resistance thermometer. On June 18th the temperatures recorded for one position were: surface 19.27°C., average for 1 to 15 meters, 18.9°; 20 meters, 15.8°; 25 to 35 meters, 12.8°. The surface temperature reached a maximum of 29.3° on July 16th while the bottom rose to 14.0°. The 13 to 18 meter layer remained almost constant, but a thermocline was developed at 8 to 11 meters (July 2nd). The thermocline was found at successively greater depths until it reached 17 to 18 meters (August 18th). On this date the 1 to 3 meter layer was 23.8°, and the bottom was 14.72°.

Lake East Okoboji is about 5 meters deep, and the surface-bottom difference seldom exceeded 1° except on days of dead calm and bright sunshine. The highest surface temperature recorded was 30.75° on July 16th, and the highest average for 0 to 5 meters was 28.93° on July 18th. The average for all depths of East Lake was always higher than for the 0 to 5 meter layer of West Lake.

220. DISSOLVED OXYGEN CONTENT OF THE OKOBOJI LAKES, SUMMER 1936. George E. Jay, Jr., Iowa Lakeside Laboratory and State University of Iowa. (Introduced by R. L. King.)

Measurements of the dissolved oxygen content of the Okoboji Lakes were made at 2- to 4-day intervals during July and part of August, 1936, by means of a modified Winkler method. The oxygen content of the epilimnion of West Okoboji was evidently dependent to a large extent upon wind action, and the measurements varied from 2.92 to 6.12 cc./liter (approx. 55% to 95% saturation). Samples from 25 to 35 meters varied in oxygen content between 0.7 and 1.11 cc./liter until August 2nd when it increased to 1.70 to 2.35 cc./liter. About August 12th there occurred a sudden decrease to about 0.28 cc./liter which was maintained as long as readings were taken. After August 12th an odor of H_2S was strongly evident in the bottom waters. These measurements indicate a very late stagnation period for the bottom waters which is rather unusual for a lake of this depth (compare temperature data elsewhere).

The oxygen content of East Okoboji varied from 1.15 to 8.59 cc./liter (approx. 21% to 137% saturation); differences between surface and bottom (5 meters) seemed to depend upon the total amount and vertical distribution of algal growth. The oxygen content showed a much wider range of variability than the upper 5 meters of West Okoboji. The algal growth in East Lake is much greater than in West Lake, and the large variation in oxygen content was probably caused by changes in the relative amounts of growing and of decaying algae.

221. A PRELIMINARY NOTE ON THE ECOLOGY OF THE PSAMMON OF SOME WISCONSIN LAKES. Robert W. Pennak, University of Wisconsin. (Introduced by C. Juday.)

Quantitative and qualitative studies of the microscopic fauna of sandy beaches of thirteen Wisconsin lakes indicate that this environment harbors a characteristic biota.

Harpacticoid Copepoda were concentrated in narrow zones in the sand; one station on Lake Mendota, for example, showed a population of eighteen individuals per 10 cc. of sand, 180 cm. from the water' edge; stations nearer and further away from the water showed about one individual per 10 cc. Vertically, Harpacticoids were most numerous at depths of 2 and 3 cm. Few Harpacticoids were found on shores of soft-water lakes.

Tardigrada also showed horizontal population stratification, but were most abundant on the shores of soft-water lakes.

Numerically, the Rotatoria formed the bulk of the psammon. The great majority of them belong to the Colurinae, Lecaninae, Dicranophorinae, and Notommatidae. The distribution of the various species appears to be a complex problem. Some species, like Monostyla hamata Stokes, are abundant at many locations while others seem restricted to certain shores. Also, in some places a definite vertical and horizontal distribution is evident. Several undescribed species were found.

Gastrotricha, Oligochaeta, Turbellaria, insect larvae, and Nematoda were often present but in small numbers.

The chemical and physical data collected show that the distribution of the various forms is correlated to a considerable extent with such factors as: slope and width of the beach, amount of organic detritus present in the sand, temperature, chemistry of the lake, and the amount of capillary water held in the sand.

222. SCIENCE IN FISH CULTURE. II. WITH SPECIAL REFERENCE TO BIOECOLOGICAL HATCHERIES OR REARING STATIONS. H. P. K. Agersborg, National Park Service.

A springfed brook in Ossipee, New Hampshire, 3332 feet long and with a more or less constant temperature varying between 50 to 55°F. and a minimum volume of water flowage of 150 gallons per minute was conditioned for 615,000 salmonids of which 3500 were 1½-year-old rainbow, 3000 1½-year-old brook trout 6 to 10 inches long, 150,000 chinook 3 to 4 inches, 90,000 lake trout 2 inches, and 368,500 younger brook trout varying in size from 1½ to 5 inches. The fish were segregated in compartments according to size and species; the lake trout in the least turbulent water. Sixteen compartments in all were built. The banks overhung with myrtle were not disturbed. All vegetation except for a trail to make feeding possible was left intact. The mud which originally was in a living balance and composed of decaying vegetation but alive with diatoms, desmids, worms, crustacea, and insect larvae of many kinds was removed from the lower half of the brook to save oxygen if the mud should die. The bottom of the brook was perfectly smooth with a gradual gentle slope and consisted for the most part of diatomaceous earth; the rest was white sand. In the upper part of the brook where the mud could be left, the trout grew the fastest. A marshy area about 1570 square feet in the middle upper half of the stream was left as an unused reservoir. Much natural food came from it. The cost of construction of this rearing station was 180 times less than the cost of a similar station built by the State without bioecological guidance.

223. FOOD AND FEEDING ACTIVITIES OF THE SALT MARSH SNAIL (MELAMPUS BIDENTATUS). Sibyl A. Hausman, Connecticut College for Women. (Introduced by Pauline H. Dederer.)

In 1932 (Am. Nat., vol. 166, November to December, p. 541) the writer recorded some observations on the ecology and principal food elements of the salt marsh snail (Melampus bidentatus). Since that time, continued observations in the field and studies of captive individuals in the laboratory, collected in the vicinity of New London and New Haven, Connecticut, have shown that Melampus also feeds upon decaying fish and other animal tissues. It is estimated that the proportions of the principal food elements in the dietary of this species are approximately as follows:

Algae (chiefly diatoms)	75%
Tissues of higher plants	15%
Decaying animal tissue	10%
Inorganic substance	trace

Melampus feeds at night, but especially during the morning and evening twilight hours, or on dark, humid days; retiring when the sun is high and the air is dry to sheltered situations—as underneath stones, shells, masses of marsh grass, or crevices and cavities in the mud. During the winter they hibernate in similar situations, but hibernation is only partial. During warm periods they emerge and feed actively.

224. STUDIES IN POPULATION PHYSIOLOGY. VII. THE RELATION OF ENVIRONMENTAL CONDITIONING TO THE DECLINE OF TRIBOLIUM CONFUSUM POPULATIONS. Thomas Park and Nancy Woollcott, Johns Hopkins University.

An analysis was made of the effect of 5%, 10%, 15%, 20%, 25%, 50%, 75% and 100% conditioned flour (flour taken from senescent Tribolium cultures) on the fecundity and fertility of the beetle, Tribolium confusum. It was shown that, in general, the rate of egg production varied in inverse ratio with the conditioned flour content of the medium. The fertility, on the other hand, was not appreciably altered by such differential conditioning. These physiological relations are discussed as factors contributing to the decline of Tribolium populations.

225. REGIONAL DISTRIBUTION OF NORTHEASTERN TEXAS ACRIDIANS. F. B. Isely, Trinity University (Texas).

In making a field study (1931–1936) of the regional distribution of eighty-nine acridian species within northeastern Texas, it was found that these grasshoppers may be grouped into three different acridian faunal belts. These acridian belts closely parallel combinations of the five soil-floral belts that are marked off across northeastern Texas.

The east Texas timber acridians are made up chiefly of eastern and southeastern species and those of wide general distribution.

The blackland prairie grasshoppers, on the other hand, in addition to the species of wide general distribution, receive important new elements from the west and the southwest. The blackland prairies may be designated as a delimiting belt marking the extreme southeastward advance of twenty-four great plains species as well as the westward advance of six east Texas timber species.

Through the grand prairies several additional great plains species deploy sparingly, but they are not found to breed in the blackland prairies. Northeastern Texas grasshoppers apparently find their optimum living conditions in the grand prairies; here develop the population peaks both as to number of species and aggregate numbers of individuals in several of the dominant species.

Miscellaneous

226. COPULATION IN THE ACOELOUS TURBELLARIAN AMPHISCOLOPS LANGERHANSI. Libbie H. Hyman, American Museum of Natural History.

Copulation has never been observed in the Turbellaria acoela but may be presumed in most species from their possession of a penis. In tanks at Bermuda copulation was repeatedly witnessed in Amphiscolops. Two individuals on meeting indulge in a brief sexual play, consisting of a few nips at each other, and then suddenly roll up into a ball. The outer animal is so curled around the inner one that the ventral surface of its posterior end is in contact with the same surface of the rear end of the other. After attachment, the animals unroll and remain motionless with ventral surfaces against the glass, heads more or less away from each other. Connection lasts 45 to 50 minutes and is so firm that couples may be fixed in copulation. Sections of such couples show that the penes of the partners are dove-tailed together and a stream of sperm passes from each worm over the anterior edge of the partner's penis into the seminal bursa of the partner. The opening into the seminal bursa lies just anterior to the penis. This type of copulation appears to be unique among animals. The eggs are laid about midnight as a flattened gelatinous mass containing four to fourteen, mostly seven to ten, eggs and hatch in about 5 days to complete worms. Some observations on early cleavage indicate disagreement with the standard account. There is no definite arrangement of the blastomeres into micromeres and macromeres.

227. Some cave planarians of the United States. Libbie H. Hyman, American Museum of Natural History.

To the well-known eyeless triclad of the Kentucky caves, Sphalloplana percoeca (Packard) 1880, may now be added four new species of American cave planarians. One of these, Phagocata subterranea, from Indiana caves, is polypharyngeal, has a pair of eyes at least when young, and does not differ from epigean species of Phagocata. The other three are eyeless and display a peculiarity unique among triclads—an anterior adhesive organ in the form of an eversible deeply invaginated gland operated by a strong retractor muscle. In Speophila buchanani, Mammoth Cave, the anterior end containing the gland is not delimited from the body; in Sp. pricei, Pennsylvania caves, this region is sharply narrowed from the broad body; and in the very remarkable Kenkia rhynchida, from an Oregon cave, the gland occupies a snout-like anterior projection from the oval deeply concave body which also lacks a postpharyngeal region. As the copulatory apparatus of these planarians conforms to that of the genus Phagocata, these worms are presumably derived from Phagocata and can be arranged in an evolutionary series beginning with the little modified Phagocata subterranea, having no adhesive organ. Next comes Sphalloplana in which the adhesive organ begins as a glandular cup in the center of the anterior margin. In Speophila this has invaginated into a deep gland and the series terminates with the strange form Kenkia where the gland occupies a snout. The gland secretes a sticky material which presumably aids in the capture of prey.

228. Studies on host reactions to larval parasites. I. Effect on weight. George W. Hunter, 3rd and Wanda S. Hunter, Wesleyan University.

While it has been supposed that a heavy infection by larval parasites detrimentally affects the well-being of the host, relatively little evidence has been presented to this effect among the fishes. The authors ran the following experiment using no. 4 fingerling small-mouthed bass (M. dolomieu) furnished by the New York Conservation Department. Thirteen fish were divided into two lots, five being kept as controls. Both control and experimental fish were marked, weighed and fed the same amount of food per gram weight of fish. Temperature conditions were kept relatively constant. The eight experimental fish were subjected to infection by the cercaria of the black grub of bass (Cercaria bessiae). In 3 months these fish showed a loss in weight of 0.17 to 1.7 gm., or an average of 0.62 gm. per fish, in fish weighing less than 10 gm. at the start of the experiment. All controls except one gained between 0.01 to 0.32 gm., or an average of 0.065 gm. per fish. Analysis by R. A. Fisher's method of the difference between the means of the two sets of observations shows that the average change in weight between the two lots of fish was significant. Infected fish harbored between 193 and 708 metacercarial cysts of the black grub (Crassiphiala ambloplitis) produced by C. bessiae. This experiment demonstrates that heavy infections with this larval tissue inhabiting parasite causes a loss in weight on the part of the host.

229. RESISTANT AND SUSCEPTIBLE GROUPS OF WHITE LEGHORN CHICKENS TO THE NEMATODE ASCARIDIA LINEATA (SCHNEIDER). J. E. Ackert, Ivan Pratt and A. E. Freeman, Jr., Kansas State College.

By selecting the most resistant cockerels and pullets from a sturdy flock of chickens, a resistant group of White Leghorns was developed. From another sturdy flock of the same breed a cockerel and six hens were taken at random. As their offspring proved to be less resistant to the roundworm Ascaridia lineata, they were called the susceptible group. The numbers and lengths of worms from the offspring (examined at the same ages) of these two groups were as follows: from the resistant group an average of fourteen worms and from the susceptible group an average of 17.6 worms; lengths, from the resistant group an average of 27 mm. and from the susceptible group, 29.4 mm.

During the next year father-daughter and brother-sister matings from the resistant group were made, giving a second generation group which produced offspring that were more resistant than were similar offspring from the susceptible group. The average numbers and lengths from these groups were: from the resistant group an average of 2.6 worms and from the susceptible group an average of 3.7 worms. In lengths those from the resistant group averaged 16.9 mm. as compared with 20.5 mm. for the susceptible group, a significant difference.

Such tests have been carried on for three generations. In the last the worms from the resistant group were significantly fewer than those from the susceptible group while the worms from both groups were of approximately the same length. This lack of difference in length of worms is attributed in part to inbreeding without continued selection.

These results give promise of ability to establish by selection a strain of chickens that is quite resistant to the fowl nematode Ascaridia lineata (Schneider) as well as one that is distinctly susceptible to it.

230. NEURONS AND CONNECTIVE TISSUE CELLS IN THE ENTERIC WALLS OF NECTURUS. Elbert C. Cole, Williams College.

In Vom Rath preparations of the enteric wall of Necturus, nerve cells closely resemble connective tissue cells. Both types of cells are to be found in the region lying between the outer and the inner muscle tunics of the digestive tract. Nerve cell nuclei are somewhat more elongated and flattened than connective tissue cell nuclei. The nerve cells of the myenteric plexus appear to lie exclusively between the muscle coats. Connective tissue cells, on the other hand, may be found also within the muscle layers, as well as in the submucosa. This conforms with conditions found in the frog (Cole, '26).

In methylene blue preparations of Necturus, McKibben ('14) described cells in the meninges that might easily be mistaken for nerve cells. These are the same type of cells that the present writer has observed not only in the meninges but also generally distributed throughout the enteric wall of Necturus, following treatment with methylene blue.

It has been shown (Cole, '25) that connective tissue cells and nerve cells in the frog's digestive tube can be differentiated by their reaction to methylene blue, used supravitally. The same thing appears to be true with respect to homologous cells in the digestive tract of Necturus. Thus physiological differences as well as structural and distributional differences may be used in identifying these types of cells.

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ERRATA

The title for abstract 95 on page 24 of Supplement no. 1, The Anatomical Record, vol. 67 no. 1 should read:

95. Natural duration of larval life in Phallusia nigra. (15 min.) (Lantern.) Caswell Grave and Paul A. Nicoll, Washington University.

The title for abstract 95 on page 71 of Supplement no. 1, The Anatomical Record, vol. 67, no. 1 should read:

95. NATURAL DURATION OF LARVAL LIFE IN PHALLUSIA NIGRA. Caswell Grave and Paul A. Nicoll, Washington University. (Lantern; 15 min.)

VACUOLATION OF THE ANTERIOR PITUITARY GLAND FOLLOWING CASTRATION, IMPLANTATION OF CANCER TISSUE AND THYROIDECTOMY[1]

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TWO PLATES (TWELVE FIGURES)

In an earlier paper (Guyer and Claus, '32) the authors showed that transplanted rat carcinoma after a period of growth in a susceptible animal induces a vacuolation of the basophile cells of the anterior pituitary gland that is indistinguishable from that which follows castration. Furthermore, a subsequent study ('34) failed to reveal any characteristic differences in the Golgi apparatus of these two types of cells—an observation deriving its significance from the fact that the vacuolar substance appears to arise as a secretion within the Golgi framework or to be transformed by it. Inasmuch as thyroidectomy is also followed by the formation of vacuoles in pituitary cells it became of interest to compare the two series of results. A careful cytological study of the basophile cells in the anterior hypophyses of many experimental animals has convinced them that the vacuolation caused by thyroidectomy is of a different type from that which follows castration. A brief report was made on this material at the annual meeting of the American Association of Anatomists in April, 1935 (Abstract, Anat. Rec., vol. 61, no. 4, Supplement, p. 21). Further study of the original material, together with that of additional sections from numerous castrated and cancerous animals is reported in the present

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paper. In our opinion the evidence justifies the inference that the vacuolation which follows thyroidectomy is a degenerative phenomenon, probably hydropic in nature, and not comparable to that resulting from castration. The latter type has the appearance of being secretory, as has that induced by transplanted carcinoma, except in very late stages of malignancy when in moribund animals the degenerative type of vacuolation may appear.

In a careful study of many hundreds of vacuolated basophile cells in the anterior pituitary glands of twenty-five castrated rats the vacuolation has been found almost invariably to be closely associated with the Golgi apparatus as described and pictured in our 1934 paper. The condition is shown photographically in the present paper in figure 5. When one studies the vacuolation following thyroidectomy, however, not only are the vacuoles seen to be characterized by apparently thinner consistency but there is no regular association of the vacuole with the Golgi apparatus. The two are just as likely to be far apart in the cell as to be in juxtaposition. The Golgi apparatus in such cells, indeed, is often in a scattered diffuse condition instead of the network characteristic of the normally functioning basophile cell and of the early castrate basophiles. In thyroidectomized rats, it seems to be rather in process of disintegration than to be acting in a secretory capacity. In other words, the hypertrophy of the basophilic cells concerned appears to be due largely to the imbibition of liquid, probably water; and the Golgi apparatus together with various other cell contents seems to be undergoing dissolution.

Furthermore, in our preparations, wherever vacuole, nucleus and Golgi body are visible in the same section of a basophile cell, while no regular relationship of the nucleus to either the vacuole or the Golgi apparatus is observable, it never, according to our observations, lies between the two. It is not uncommon, however, for the Golgi body to lie between the nucleus and the vacuole (fig. 5). That is, the Golgi apparatus seems always closely associated with the vacuole in the vacuolated basophilic cells of castrates. We have been able to find no such constancy of relationship in the basophiles of thyroidectomized rats. In figure 4, for instance, which is a photograph of a section from the anterior pituitary gland of a thyroidectomized rat, the nucleus of a vacuolated basophile is visible directly between the Golgi complex and the vacuole. In figure 5 is seen a similar cell from a castrated animal with the nucleus to one side, and the usual juxtaposition of Golgi apparatus and vacuole found in castrates or in cancer implanted animals.

Again, it is common to find more than one large vacuole in the basophiles of the thyroidectomized animals. The divided nature of the vacuole which is frequently seen in such rats, in fact, was the first thing that led us to suspect that we were dealing with a kind of vacuolation that differed from that found in the basophiles of castrated animals. This multiple condition is shown in the lower, central region of figure 4. Sometimes as many as from three to five large separate vacuoles may be seen in one cell. Moreover there is often what appears to be a breaking down of such cells since it is not uncommon to find areas of wall-less, disintegrating protoplasm in the midst of cells with walls still intact.

Another difference that seemed fairly constant was the rather scattered distribution of the vacuolated cells in the cancer implanted or in the castrated rats, as contrasted with more of a grouping of such cells in the thyroidless animals. And while in the former the nucleus was usually to one side of the vacuole, in the latter it was not uncommon for the vacuole to enclose the nucleus. The nuclei themselves, indeed, in the thyroidectomized animals, often showed vacuolation, a condition we have not observed in castrated animals.

Again it was not uncommon to find vacuolated oxyphile cells in the thyroidectomized rats and such a condition in the oxyphiles of castrated animals was rarely encountered, even though our studies covered a greater number of animals of the latter type.

Then, too, various physiological tests bear out the view that the changes brought about in the anterior pituitary cells by castration are not the same as those which result from thyroidectomy. Thus, anterior lobes of pituitary glands, transplanted from rats some months after castration, induced marked precocious sexual development in twenty test animals; the same was true of similar tissue from rats with well-developed transplanted carcinoma. On the other hand, similarly transplanted anterior lobes from thyroidectomized rats induced no more precocity in sexual development than anterior pituitary glands of normal rats.

If, as one might suppose, the pituitary vacuolation in thyroidectomized animals is not a phenomenon peculiar to the pituitary gland but is rather the general result of insufficient oxidation, then evidence of similar injury might be expected in other organs of the body. Furthermore, one would expect such injury to be most apparent in particularly active organs, such as the liver, heart and adrenals. With this thought in mind these organs from the thyroidectomized rats were carefully studied. The cells of the liver in particular were found to be markedly altered; they were profusely vacuolated. Figures 7, 10 and 12, are photographs showing respectively the appearance of normal rat liver cells, of liver from a thyroidectomized rat, and lastly of liver from a thyroidectomized rat which had subsequently been fed powdered thyroid to restore normal oxidative conditions. The cells of the latter are obviously much like those of normal rat liver. The liver cells of the castrated rats (fig. 2) were not demonstrably different from those in normal rats (fig. 1). The Golgi apparatus of the liver cells in the untreated thyroidless animals (fig. 3) was of a diffuse and sometimes of a granular type, apparently in process of dissolution, while in the liver cells of castrated rats, this apparatus remained mostly of the continuous or mesh-like form. Figure 11 shows a section, viewed under relatively low power, from a thyroidectomized guinea pig in which the vacuolation of the liver cells is even more extreme than that seen in the rat. In the liver of the guinea pig, however, the appearance is more that of fatty than of hydropic degeneration. Both the heart tissue and the adrenals, of the thyroidectomized rats also showed vacuolation though not as extensively as the liver.

In rats implanted with carcinoma the basophile cells of the anterior pituitary gland maintain, in general an appearance similar to that of castrated animals. Most of the cancerous animals were killed while they were still active and not in the last stages of malignancy. In a few of the latter, however, killed when it was evident that they were moribund, the condition of vacuolation in the anterior pituitary cells often took on more the appearance seen in the corresponding cells of thyroidless rats. The cells seemed to be on the point of dissolution, the Golgi apparatus was broken and scattered, and instances were found in which the nucleus might lie between it and the vacuole or vacuoles, as in thyroidectomized animals. This would seem to indicate that in the basophiles of such moribund animals, a type of hydropic degeneration was replacing the earlier secretory vacuolation.

From the foregoing facts the authors infer that the vacuolation seen in the anterior lobe basophiles in the castrated rats, and in cancerous rats that are not yet in the final stages of the malady, is probably secretory in nature, while that of such cells in thyroidectomized rats and carcinomatous rats in a moribund state is more likely due to hydropic degeneration following inadequate cellular oxidations. Certain it is that the thyroid is intimately concerned with the oxidative processes of the animal body, and its removal must profoundly affect internal oxidations. Perhaps the further inference is warranted that an insufficiency of oxygen is followed by an acidic condition of the cell protoplasm and that such acidosis renders the protoplasmic colloids sufficiently hydrophylic to induce an inward flow of water through the cell wall and its subsequent accumulation in the form of vacuoles. If this interpretation is correct the vacuolation which follows thyroidectomy is obviously different from the seemingly secretory type which results from castration. Hohlweg and Junkmann ('33) on still different lines of evidence have likewise concluded that the hypophyseal vacuolation after thyroidectomy differs from that which follows castration.

SUMMARY

In castrated rats	*In thyroidectomized rats*
1. Vacuoles of anterior pituitary basophiles are closely associated with the Golgi apparatus.	1. Vacuoles are not regularly associated with Golgi apparatus.
2. The nucleus is not found between the Golgi apparatus and the vacuole.	2. The nucleus may be between the Golgi apparatus and the vacuole.
3. The Golgi apparatus is usually a network.	3. The Golgi apparatus is commonly granular or diffuse.
4. Generally a large single vacuole occurs in the basophilic cell.	4. Frequently multiple vacuoles occupy the basophilic cells.
5. The vacuolated basophiles are usually scattered.	5. The vacuolated basophiles are commonly grouped.
6. The vacuole is usually to one side of the nucleus.	6. The vacuole often surrounds the nucleus.
7. The nuclei are not vacuolated.	7. The nuclei are commonly vacuolated.
8. The oxyphiles are rarely vacuolated.	8. The oxyphiles are often vacuolated.
9. Only occasional basophile cytolysis occurs.	9. Frequent basophile cytolysis occurs, forming vacuolated intercellular masses.
10. The cells of the liver are as non-vacuolated as in normal rats; likewise for the adrenals and the heart.	10. The cells of the liver show profuse vacuolation; likewise for the adrenals and the heart.
11. The Golgi apparatus of the liver cells is usually a network.	11. The Golgi apparatus of the liver cells is of a diffuse, granular type.

12. The transplanted anterior lobe of the pituitary gland induces marked precocious sexual development.	12. The transplanted anterior lobe induces no more precocity in sexual development than does anterior pituitary glands of normal rat.

From the foregoing facts it is inferred: 1) That the vacuolation of anterior-lobe basophiles in castrates is probably secretory in nature; 2) that in thyroidectomized animals it is probably a matter of cellular degeneration.

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PLATE 1

EXPLANATION OF FIGURES

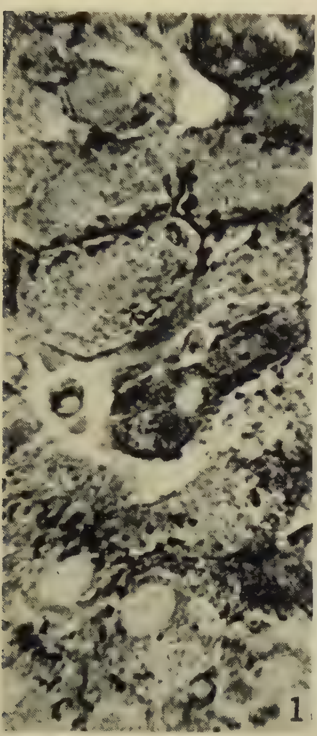
1 Photograph of cells from the liver of a normal rat showing the Golgi apparatus (in black). The cells were treated to bring out the Golgi materials but otherwise unstained, hence the nuclei appear more or less clear. × 970.

2 Photograph of cells from the liver of a castrated rat showing continuous nature of the Golgi apparatus. × 970.

3 Photograph of cells from the liver of a thyroidectomized rat showing the Golgi apparatus scattered and diffuse. × 970.

4 Photograph of basophilic cells from the anterior pituitary of a thyroidectomized rat showing multiple condition of the vacuole within a single cell. Several cases of the nucleus lying between the vacuole and the Golgi apparatus are also shown. *n*, nucleus; *g*, Golgi apparatus; *v*, vacuole. × 970.

5 Photograph of basophilic cells from the anterior pituitary of a castrated rat showing the usual picture of one large vacuole in a cell with its closely associated Golgi apparatus. The nucleus may lie at one side of or beyond the Golgi complex but not between it and the vacuole. *n*, nucleus; *g*, Golgi apparatus; *v*, vacuole. × 970.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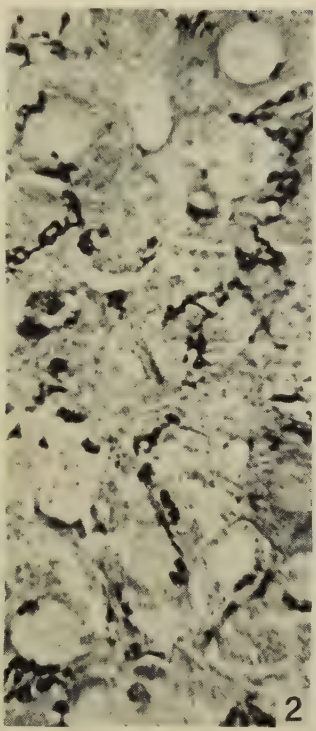
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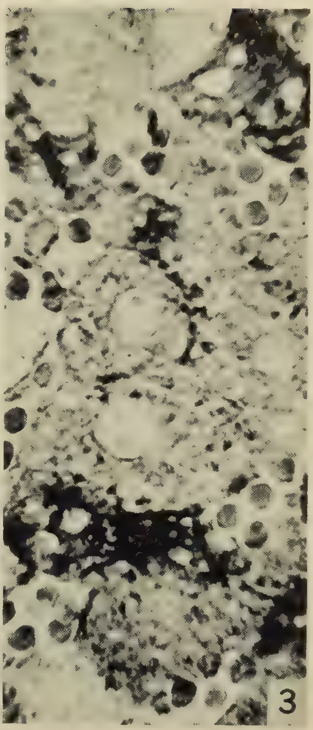
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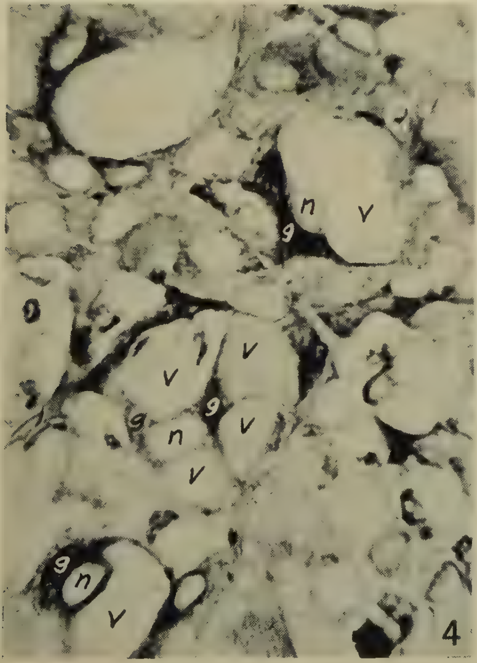
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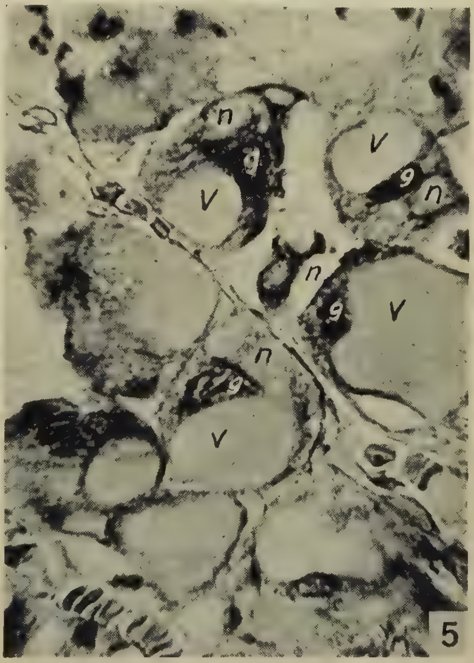
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PLATE 2

EXPLANATION OF FIGURES

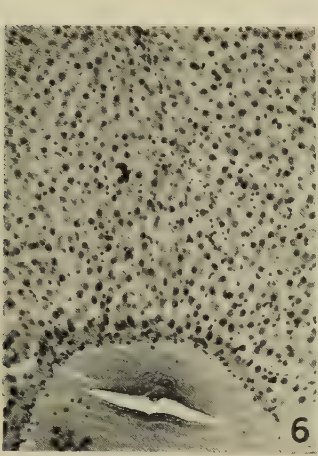
6 Photograph of cells from the liver of a normal rat. × 100.

7 and 8 Photographs of cells from the liver of a normal rat. The differences in appearance are due to differences in staining. × 430.

9 and 10 Photographs of cells from the liver of a thyroidectomized rat, × 100 and × 430 respectively, showing vacuolation of cells.

11 Photograph of cells from the liver of a thyroidectomized guinea pig. Extreme vacuolation is manifest. × 100.

12 Photograph of cells from the liver of a thyroidectomized rat which subsequently had been fed powdered thyroid. The cells have been restored to almost but not quite the normal appearance as seen in figure 7, which shows a section similarly stained. × 430.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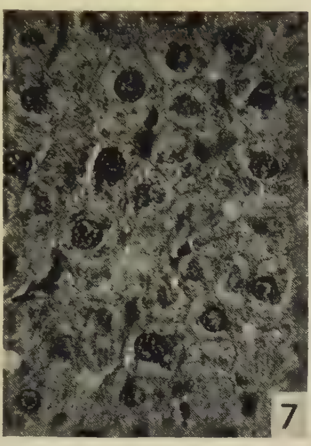
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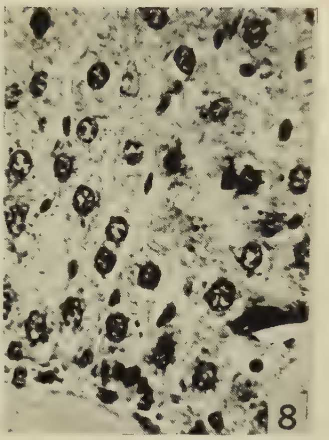
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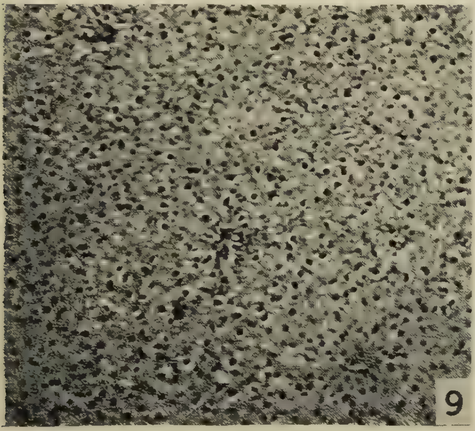
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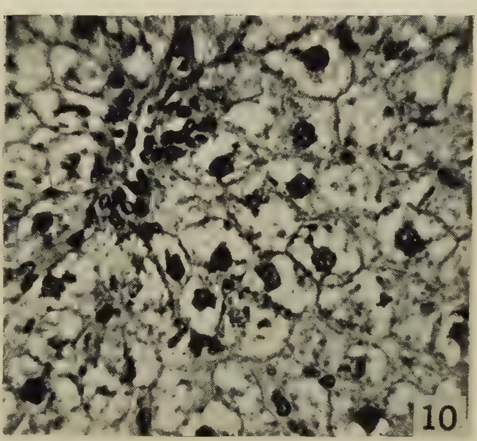
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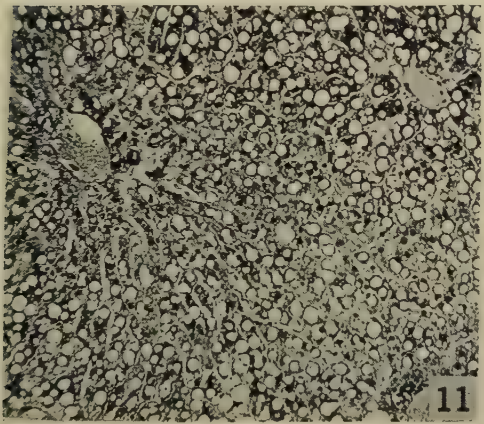
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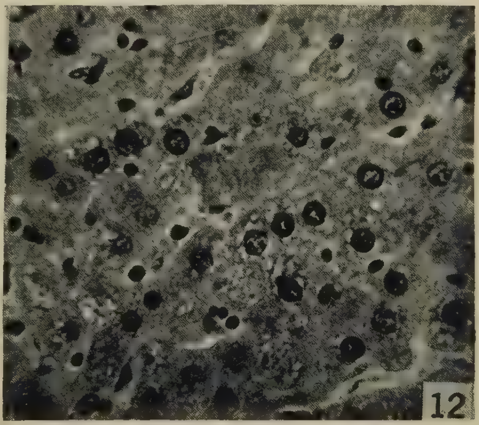
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A THORACOPHAGUS, ONE WITH HARELIP AND CLEFT PALATE

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ONE FIGURE

The literature on conjoined twins is extensive. The papers that are obtainable here show no similarity to the one which will be reported. The twins are female, joined together from the suprasternal notch to the umbilicus. The umbilical cord is single but appears as the fusion of two cords; at the placental end they are separated for a distance of 40 mm. with amnion left between. The peculiarity of this monster is that one of the twins has harelip and cleft palate while the other has a normally developed face. The deformity appears on the left side of the face, the nostril of that side is deformed by the forward and medial inclination of the left medial incisor. On opening the abdomen the livers are found joined together.

From measurement the twins are nearly equal in size, none can be called the parasite of the other.

Newman ('24), from his study on Texas armadillo, believes that the failure of the closeness of resemblance between monozygotic human twins is due to the segregating of unit characters by somatic divisions. The result is the inequality of distribution of the determining factors during cleavages. This specimen supports his explanation because harelip and cleft palate are inherited and have been known to occur in several members of the same family as cited by Ballantyne ('04). Reed and Snell ('31) have shown that harelip in mice is inherited as a recessive mendelian character. Reed ('33) on his further investigation of the embryology of harelip and cleft palate in the same species of animal concluded that the abnormality is due to the retarded growth rate of portions of the maxillary processes subjected to a localized effect of a gene. He then considers direct heredity as the main factor in producing this defect upsetting all the other theories quoted in his paper. Following this theory, if there is no secondary factor involved in the development of these monozygotic twins, both faces ought to have harelip and cleft palate. The somatic divisions, therefore, are the determining factor in the development of the different characteristic features of the twins developed from a single ovum as shown in this specimen.

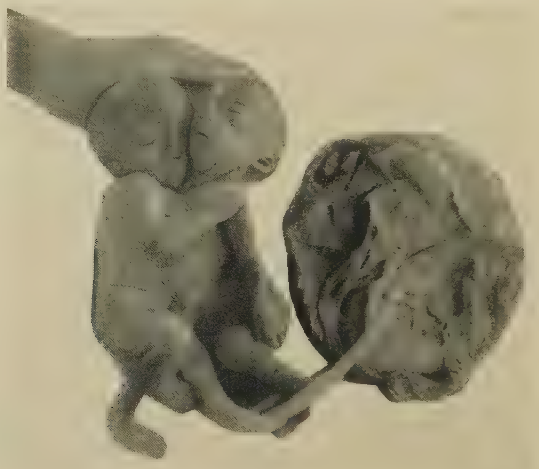

Figure 1

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HYPOPHYSEAL GONADOTROPIC HORMONES AND THE LUTEINIZATION PHENOMENON IN THE RAT

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ONE PLATE (FOUR FIGURES)

INTRODUCTION

The question of the number of gonadotropic hormones of the hypophysis is still one of great importance. Riddle, Bates, Lahr, and Moran ('36) reported that it is the follicle stimulating hormone, as measured by the effect on the immature dove testis, that is responsible for ovulation in the rabbit and that no separate hormone need be postulated to account for this phenomenon. However, Fevold et al. ('31, '33, '34) have, with their purified extracts, given rather conclusive evidence of two distinct gonadotropic hormones (follicle stimulating hormone and luteinizing hormone). Yet the discovery of Fevold and his associates ('34) of the augmentation action between follicle stimulating hormone and luteinizing hormone and the discovery of many augmenting substances, the most important of which are some of the inorganic salts, first discovered by Maxwell ('34), have led many investigators to believe that the two physiological reactions of follicle stimulation and luteinization are simply a difference in dosage of a single gonadotropic hormone, and that the luteinizing hormone acts only as an augmenter.

The continuous growth of follicles in the ovary of female rats with masculinized hypophysis (Pfeiffer, '36 a) or the reaction of the ovarian graft in the male (Goodman, '34 and

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Pfeiffer, '36 a, '36 b and '36 c) has not been explained by the theory of a single gonadotropic hormone. There is sufficient follicle stimulation but there is no hormone for luteinization or at least it is not released from the hypophysis in sufficient amounts to cause luteinization of the ovary under its influence. In parabiosis (Witschi and Levine, '34) it is shown that follicles may be over-stimulated without luteinization. Any of these ovaries can be luteinized by the injection of luteinizing hormone.

Fevold ('35) has shown that copper salts are the most effective of the metallic salts in augmenting the gonadotropic hormones. Therefore, copper sulfate has been used in the following experiments which consist of an investigation of the effects of metallic salts upon the endogenous gonadotropic hormones, the evidence for two distinct hormones, and the distribution of the two hormones in the sexes.

MATERIALS AND METHODS

Albino rats from a single commercial strain were used in all experiments. The females with masculinized hypophyses were prepared according to the technique of Pfeiffer ('36 a). The normal females were 120 to 150 days of age and were littermates of those with masculinized hypophyses. The males were 85 days of age when the ovaries were grafted in the eyes (after the technique of Goodman, '34), and were injected with copper sulfate or the hypophyses were used for transplantation 1 month later.

A 10% solution of copper sulfate was used. The dose was divided into eight aliquot parts and injected twice daily for 4 days. Biopsy or autopsy was performed at the end of the fifth day. For the augmentation effect of the copper salt, the luteinizing hormone was suspended in the copper sulfate solution and injected in the same manner as the copper sulfate alone. The luteinizing hormone was prepared from acetone dried sheep anterior pituitary powder, according to the technique of Fevold et al. ('33), suspended in distilled water and standardized on females with masculinized hypophyses.

In the males the ovarian grafts were observed with a binocular microscope during the period of injection and then removed for histological study. All sections were cut at 8 μ and stained with Delafield's hematoxylin and eosin.

RESULTS

Injection of copper sulfate and lutenizing hormone into constant oestrus females. Constant oestrus females (with masculinized hypophyses) had been used for standardization of the luteinizing hormone and it was ascertained that four daily injections of 0.5 mg. of luteinizing hormone was the minimal luteinizing dose. Therefore, to test the augmentation of the copper sulphate, six constant oestrus females were given the minimal dose of luteinizing hormone and six the same amount of luteinizing hormone in combination with 20 mg. of copper sulfate. The luteinizing hormone alone caused ovulation and luteinization of only a few of the follicles, and produced complete dioestrous smear in the vagina of only half the animals. However, the combination of luteinizing hormone and copper sulfate produced complete ovulation and corpora lutea formation. In every case the smear reached the dioestrous stage within 3 days from the first injection and returned to oestrus after 4 days (table 1).

TABLE 1

Injection of luteinizing hormone and copper sulfate into constant oestrous females

NUMBER OF ANIMALS	DAILY INJECTIONS		TOTAL INJECTIONS		LAPAROTOMY ON THE FIFTH DAY, OVARY	DIOESTRUS BEGINS AFTER FIRST INJECTION	DURATION OF DIOESTRUS
	L H	$CuSO_4$	L H	$CuSO_4$			
	mg.	*mg.*	*mg.*	*mg.*		*days*	*days*
6	0.5		2.0		A few corpora lutea	2–3	3
6	0.5	5.0	2.0	20	Corpora lutea	2–3	4
12		5.0		20	Only follicles	3–4	1+

Examination of the ovaries was made under the dissecting microscope at the time of biopsy, that is on the fifth day. The ovaries were not removed for histological study since this phase of the problem has already been described by Witschi and Pfeiffer ('35).

Twelve females (with masculinized hypophyses) which had been in constant oestrus for 2½ to 3 months were used to test the effect of copper sulphate alone. Each received 5 mg. of copper sulfate daily for 4 days, and were biopsied at the end of the fifth day. In every case (table 1) the continuous oestrus was interrupted 3 to 4 days after the first injection. However, the dioestrus never persisted over 36 hours and continuous dioestrus was again established. Biopsy at the end of the fifth day demonstrated ovaries with continuous follicular development and without any indication of either ovulation or corpora lutea formation.

Copper sulfate injected into constant oestrus females in combination with luteinizing hormone augmented the luteinizing action, but if given alone neither produced luteinization nor augmented the follicle stimulation that was normally present. Its only action was a temporary disturbance of the vaginal smear, probably due to the toxic action of the copper sulphate. There was a definite loss of weight and within 10 days necrosis was found at the site of the injection.

Copper sulfate injected into normal females. Nine normal females were injected with 20 mg. of copper sulfate and three were used as controls. Experimental and control animals were littermates. They were the same age and in a few cases littermates of the females with masculinized hypophyses used in the previous experiment. Injections were made twice daily (2.5 mg.) for 4 days and biopsy performed at the end of the fifth day. The ovaries from four animals were removed for histological study. Macroscopical observation demonstrated the presence of about three sets of corpora lutea but not excessive luteinization. Histological study confirmed these findings.

The cycles of the remaining five animals were followed for 3 weeks. There was usually a brief disturbance in the vaginal cycle at the time of the last injection. If the female was in dioestrus or the beginning of the nucleated cell stage, at the first injection, the normal cycle followed. However, the dioestrus which appeared by the fourth or last day of injection was usually prolonged. If the female was in oestrus leucocytes appeared, but the next stages contained cornified cells, and dioestrus was always observed by the fifth day after the first injection. The cornified cells probably represented an abortive cycle. In four of the animals the next cycle had started by the end of the fifth day and all but one of the others had started within 7 to 8 days from the first injection. This animal gave a dioestrous vaginal smear for 8 days and then, as in all other cases, ran normal cycles. Biopsy at the end of the prolonged dioestrus showed ovaries with only slightly greater luteinization than its littermate control. About three sets of corpora lutea were present.

As in the case of the constant oestrus group, the disturbance in the cyclic function of the ovary was probably due to the toxic action of the copper salt. It also seemed in the single case of prolonged dioestrus that the follicle stimulating hormone had been suppressed but that the luteinizing hormone continued to act for a time, at least. There was do indication that copper sulfate in the doses given augmented the gonadotropic hormones secreted within the body.

Copper sulfate injected into males bearing ovarian grafts. Six normal and six castrated males received ovarian grafts in the anterior chamber of the eye at 85 days of age. The donors were 21 days old. After 30 days both the castrated and non-castrated groups were injected with 2.5 mg. copper sulfate twice daily for 4 days and autopsied at the end of the fifth day. Until the time of the injections the grafts were observed daily under the binocular microscope and showed only follicles. There was no change in the ovarian grafts during the injection or until autopsy. However, these observations were checked by histological study after autopsy.

The grafts varied in development from those containing only a few follicles to those with many well-developed follicles (fig. 1). In every respect they resembled any normal series of ovarian grafts in the anterior eye chamber. As usual, the castrates showed better grafts. There was no effect of copper sulfate, either upon the ovarian graft or the male accessory sexual organs. This illustrated that, as in the case of the females, the copper salt, at least in the doses given, did not cause any augmentation of the action of the endogenous gonadotropic hormones, nor did it cause the formation of corpora lutea in the presence of large amounts of follicle stimulating hormone.

Determination of the amount of luteinizing hormone in the hypophysis of the male. The male hypophysis does not normally cause the luteinization of follicles in an ovarian graft regardless of whether the testes are present or the animal is castrated after puberty. Further, oestrin cannot 'call out' the luteinizing hormone in sufficient amounts to cause luteinization of these grafts. However, Evans et al. ('35) and Lipschütz ('33) have reported that a small amount of luteinizing hormone is present in the male hypophysis. Therefore, it is important to find out how much is stored in the male hypophysis when an ovary is present. This was done by transplantation of the hypophyses.

Twenty-two normal and ten castrated males were used as donors and six normal and two castrated males were used as hosts. Both the hosts and the donors had vitalized ovarian grafts in the anterior eye chamber for 30 days. The castrates were donors to the castrate hosts and the normals to the normal hosts. The implantations were spread out over 2 or 3 days and the total number varied from two to five (table 2). The hypophyses were transplanted immediately upon removal. They were placed under the skin of the ventral body wall and crushed into many fragments with the tip of the forceps to hasten their absorption.

Two hypophyses from normal males gave a definite stimulation (fig. 2) and the stimulation increased in proportion to the number of hypophyses implanted. Five hypophyses from normal males, in spite of the great follicular development (fig. 3), did not produce any indications of luteinization. However, five hypophyses from castrate donors produced definite luteinization (fig. 4). It was possible to follow these changes in the ovarian grafts by daily examination with the binocular microscope, but all ovarian grafts were removed and sectioned.

The accessory sex organs of the normal males receiving the hypophyseal grafts showed some stimulation in the case of the larger number of implants. The effects were not as definite as those observed in the ovarian grafts, since stimulating effects were very difficult to measure in the accessory sex organs of the adult normal male. Luteinizing hormone was found in the hypophysis of these castrated males but in very small amounts.

TABLE 2

Transplantation of hypophyses of males carrying ovarian grafts into males with ovarian grafts in the eye

NUMBER OF ANIMALS	HYPOPHYSES TRANSPLANTED		CONDITION OF THE OVARIAN GRAFT, AUTOPSY PERFORMED AFTOR 120 HOURS
	Daily	Total	
2	1	2	Definite increase in size, only follicular development
2	2	4	Approximately 80% increase in size, but only follicular development
2	2[1]	5	Approximately 100% increase in size, due to follicular development
2[2]	2[1]	5	Approximately 100% increase in size, many corpora lutea present

[1] Only one hypophysis transplanted on the third day.

[2] Received hypophyses from castrated males.

DISCUSSION

The experimental data presented here demonstrate that copper sulfate has no effect upon the endogenous gonadotropic hormones. Yet there is conclusive evidence (Maxwell, '34 and Fevold, '35) that copper and zinc salts augment the potency of hypophyseal extracts. These facts indicate a combination with the injected substances to form a less soluble salt which decreases the destruction and excretion of the hormone and allows a longer period of action. This theory is supported by the fact that the salts which cause augmentation (Fevold, '35) affect both the luteinizing hormone and the follicle stimulating hormone but do not alter the type of physiological reaction. However, it is not clear whether the absence of effect with endogenous hormone is due to the small amount of hormones in the blood at any one time and the rapid disappearance of the copper sulfate or, in the case of the injected extract, to combination with the associated protein complexes.

The loss of body weight and necrotic action at the injection site indicates that the disturbance of the vaginal smear and the ovarian cycle in the normal female injected with copper sulfate may be due to the toxic effects of the salt. There is evidence that the follicle stimulating hormone is without effect on the luteinizing hormone release, which gives some support to the theory (Witschi and Pfeiffer, '35) that instead of an actual stimulation by oestrin, it is the suppression of the follicle stimulating hormone that allows the release of luteinizing hormone. There appears to be an exhaustion of the luteinizing hormone in both the immature female rat with continued oestrin injection (Fevold et al., '36) and the female in parabiosis with a castrate (Witschi and Levine, '34), but it is not clear whether this is due, in the first instance, to the high concentration of oestrin or to the immaturity of the hypophysis or to the high concentration of follicle stimulating hormone in the parabiosis experiment. However, in the female with masculinized hypophysis it is not a question of exhaustion of the luteinizing hormone by either of these means because the follicle stimulating hormone and oestrin are in equilibrium and have been, in many animals, ever since puberty (Pfeiffer, '36 a). The injection of oestrin completely suppresses the follicle stimulating hormone without any luteinization of the ovaries which are not refractory as the follicules ovulate and luteinize if luteinizing hormone is supplied (Pfeiffer, '36 a and Witschi and Pfeiffer, '35).

In spite of all the evidence in this and previous investigations of the author (Pfeiffer, '36 a), the conclusion that the male hypophysis does not release luteinizing hormone in sufficient amount to cause ovulation or luteinization in the rat appears to be in opposition to much of the recent work.

Lipschütz ('34 and '36) reported that male pituitary glands transplanted into immature females produce greater luteinization than adult female pituitary glands. Evans et al. ('35) demonstrated luteinizing hormone (which is probably not released) in the castrated male pituitary gland, and Greep et al. ('36) reported that their purified follicle stimulating hormone produced proliferation of the germinal epithelium as its only effect in the male while the luteinizing hormone stimulates the interstitial elements and the male hormone thus produced acts upon the accessories.

In discussing these findings and hypophyseal physiology in general it should be kept in mind that two types of hormones are being investigated: 1) Endogenous hormones produced within the animals, and 2) injected hormones extracted from the glands, usually of a different species. It is necessary, also, to distinguish clearly between potentiality, production, and release since, as shown by Pfeiffer ('36 a), the rat hypophysis at birth has the potential ability to produce and release both follicle stimulating hormone and luteinizing hormone, a potentiality that is lost if the testes are present during the developmental stage. The present investigation confirms the conclusion of Lipschütz ('33) and Evans et al. ('35) that luteinizing hormone exists in the castrate male pituitary even though it is not released in amounts sufficient to cause luteinization of an ovarian graft. DuShane et al. ('35) have given a good example of the difference between the continued action of endogenous and injected hormones, so that it is possible and quite probable that other differences will be found. Such a difference might explain the activation of the interstitial elements of the testis by injected luteinizing hormone (Greep, '35 and Greep et al., '36) since the release of the endogenous hypophyseal luteinizing hormone is not sufficient to cause luteinization of the ovarian graft in the male rat.

In the discussion by Lipschütz and his associates of the luteinizing coefficient of the pituitary, it must be remembered that 1) there is also follicle stimulating hormone present in the pituitary, 2) the luteinizing hormone can only act when the follicles have been sufficiently stimulated with follicle stimulating hormone, 3) the male hypophysis though smaller is more active (produces greater follicle stimulation) than the female, and 4) the castrate pituitary releases large amounts of follicle stimulating hormone but contains some luteinizing hormone. These facts shown that the luteinization coefficient of the transplanted pituitary may depend upon the amount of follicle stimulating hormone that is present and, at the same time, explain the following findings: 1) The male hypophysis is more effective in luteinizing the immature rat ovary but not in ovulating the rabbit (Lipschütz, '33), where the follicles have already been developed by the animal's own hypophysis, 2) castration raises the luteinizing effect of the hypophysis (Lipschütz, '33), when parabiotic experiments prove that it is the follicle stimulating hormone that is increased (Witschi and Levine, '34), and 3) the guinea pig hypophysis (which contains very little follicle stimulating hormone) has a very low coefficient of luteinization when transplanted into the immature rat but is effective in causing ovulation in the rabbit (Lipschütz, et al., '32).

All of this reasoning is based on the assumption that there are two distinct gonadotropic hormones. It is impossible to explain in any other way the fact that the ovary of the female with masculinized hypophysis cannot be ovulated or luteinized except by injecting luteinizing hormone, although the follicle stimulating hormone producing power of the hypophysis is normal, or that the ovary of the female in parabiosis with a castrate has follicles in every stage of development from the primary to over-stimulated and atretic follicle (Witschi and Levine, '34) which never ovulate or luteinize even with the excessive stimulation of follicle stimulating hormone. The ovaries are not unresponsive since they will respond to injections of luteinizing hormone or they will ovulate and luteinize normally after the female is separated from the castrate parabiont (DuShane et al., '35). However, it is not necessary to postulate a third gonadotropic hormone (Lipschütz, '34) to cause the maturity of the follicles as these results are easily explained by the augmentation reaction (Leonard, '32 and Evans et al., '32).

Since a small amount of luteinizing hormone has been demonstrated in the hypophysis of the castrate male bearing an ovarian graft, it might seem that a greater number of transplants would demonstrate it in the hypophysis of the normal male. However, the presence of luteinizing hormone in the castrated animals might be explained by the fact that at castration the suppression of the hormone had not been complete in all cases (Pfeiffer, '36 a). The amount of luteinizer must be small, since in spite of the well-developed follicles existing in the ovarian graft in the castrate male, five castrate male hypophyses in combination with the castrate host's own hypophysis are required to cause luteinization. If luteinizing hormone is released from the male hypophysis it is usually inadequate to cause luteinization of an ovary although there may be some evidence that luteinization occasionally occurs (Moore, '19; Wang, Richter and Guttmacher, '25; and Pfeiffer, '36c). However, the ovaries either contained lutein tissue when grafted or the male was castrated at an early age (Pfeiffer, '36 c). The findings of Lane ('35) and Lane and Greep ('35) that antra formation is due to luteinizing hormone would suggest some release, as antra are readily formed in the ovarian graft in the male. But since Smith ('30) has shown that follicles occasionally proceed to antra formation in the absence of the pitutitary, it might be possible that follicle stimulating hormone alone produces all the follicular development that occurs in the ovarian graft in the male. At least it is clear that the male and masculinized female hypophysis are alike in their responses and that the physiological difference between the male and the female hypophysis is in the amount of luteinizer that they are able to release in response to the suppression of the follicle stimulating hormone by oestrin.

SUMMARY AND CONCLUSIONS

1. Copper sulphate augments the action of the injected luteinizing hormones. When the gonadotropic hormones are supplied by the animal's own hypophysis, the copper salt has no augmentative effect. The copper sulfate causes a slight disturbance of the vaginal cycle in both the normal and the constant oestrus females. This may be due to the toxic action of the copper sulphate. The endogenous follicle stimulating hormone may be suppressed but there is no effect upon the luteinizing hormone. The augmentation by the copper sulfate when it is injected with the gonadotropic hormone is probably due to the formation of a less soluble salt with the hormone or the associated protein. This reduces the rate of destruction and excretion and causes longer and more constant action of the hormone.

2. The ovaries of females with masculinized hypophyses and the ovarian grafts in males do not luteinize following injection of copper sulfate. This indicates that luteinizing hormone cannot be released from the pituitary gland of these animals as copper sulfate does not interfere with the luteinization process in the normal female.

3. Some luteinizing hormone is stored in the hypophysis of the castrate male bearing an ovarian graft but it requires the pituitary glands of five of these castrated males combined with that of the host to produce luteinizations of the ovarian graft in the eye of this host. Five pituitary glands of normal males bearing ovarian grafts are not enough to cause luteinization of the ovarian graft in the normal male. The male hypophysis is not able to release luteinizing hormone, at least in amounts sufficient to cause luteinization of an ovarian graft.

4. A single gonadotropic hormone does not explain the physiological action of the ovary under the influence of the masculinized female or male rat hypophysis. There must be one hormone for follicular development and a second one for ovulation and luteinization. The physiological difference between the male and the female hypophysis is in the amount of luteinizing hormone they were able to release.

ADDENDUM

Since this article was submitted for publication Evans et al. ('36) reported a third gonadotropic hormone in the anterior pituitary, the interstitial cell-stimulating hormone. From their description it is closely associated with the luteinizing hormone fraction and is therefore probably contained in the luteinizing hormone fraction of Hisaw and his co-workers. This being true would explain the fact that Greep ('35) obtained only interstitial cell development with their luteinizing hormone injected into hypophysectomized males, while luteinization does not occur under the influence of the male pituitary.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

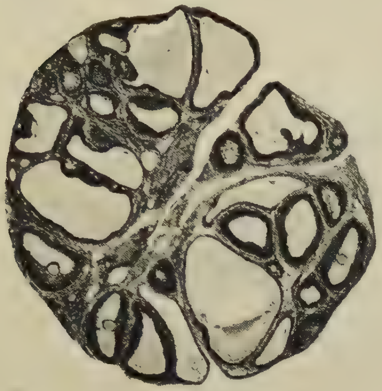
All figures are photomicrographs and taken at the same magnification

1 A cross section of a well-developed ovarian graft removed from the eye of a castrate male after 35 days. Copper sulfate had been injected 5 days previously but did not cause luteinization in spite of the well-developed follicles. × 17.

2 A cross section of an ovarian graft in the anterior eye chanmber of a normal male after receiving two pituitary glands of normal males, also carrying ovarian grafts. The graft was removed from the eye at the end of the fifth day, 35 days after transplantation. There is some follicle stimulation. × 17.

3 The same as figure 2 except that five pituitaries were injected into the host. Note the follicular development without any indication of luteinization. × 17.

4 The same as figure 3 except that the host and the pituitary donors were all castrates. Note the great number of corpora lutea, and the size of the graft in comparison to figure 3. × 17.



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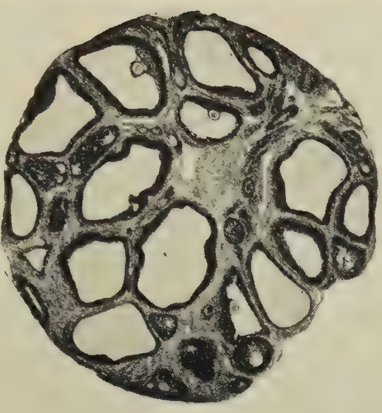

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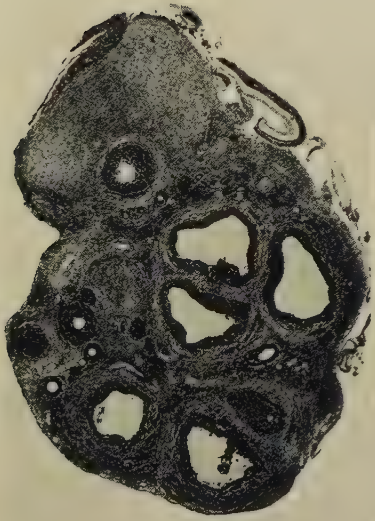

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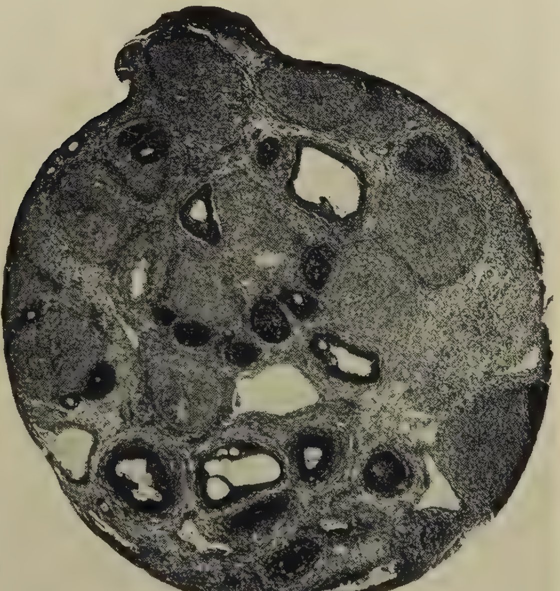

4

THE UNUSUAL MANNER OF VASCULARIZATION OF THE BRAIN OF THE OPOSSUM (DIDELPHYS VIRGINIANA)

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SIX TEXT FIGURES AND ONE PLATE (FIVE FIGURES)

In the course of a study of the blood supply of the hypothalamus and pituitary gland in a series of injected mammalian brains, the character of the cerebral capillaries in the hypopthalamic region of the opossum was found to be so unusual that it prompted us to investigate the vascular pattern of the entire opossum brain. We were rewarded by finding an arrangement of cerebral capillaries differing so fundamentally from the pattern in other mammals that it is deemed of value to describe it.

Briefly the arteries penetrating all parts of the central nervous system of the opossum are complete end-arteries and are found to terminate in individual non-anastomotic capillary loops, instead of ending in a capillary network, as seen typically in all other mammals which have been investigated. A similar type of cerebral vascular pattern has been described heretofore only in certain lower vertebrates, namely, in Lacertilia and certain urodele amphibia. The present description links the opossum in this important regard with these submammalian forms.

MATERIAL AND METHODS

The material at our disposal consists of a number of brains of the opossum (Didelphys virginiana). The blood vessels in these brains have been filled by injection through the heart with Higgins Waterproof India ink (diluted one to one with distilled water). The injection of arteries, capillaries and veins is very nearly complete. Subsequent to injection, the brains were fixed in 10% formalin, dehydrated, embedded and cut serially in alternate thick and thin sections, 200 μ and 20 μ respectively. The thick sections were mounted in balsam without staining; the alternate sections were stained in haematoxylin and eosin. One opossum was injected intravascularly, just before death, with a large quantity of 4% trypan blue solution; the brain was fixed in Susa fluid, dehydrated, embedded and cut in 200 μ sections. By this method of using trypan blue, as A. C. P. Campbell has worked out, the blood vessels of the brain can be exceedingly well delineated.

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OBSERVATIONS

Before describing the unusual disposition of the cerebral vessels in the opossum, it will be helpful to the reader to recall briefly the general pattern of the cerebral capillaries existing in other mammalian brains which have been investigated. The work of Pfeifer ('28) on the cerebral vessels of the cat serves well to illustrate the accepted ideas about the pattern of the finer blood vessels supplying the mammalian brain. It is amply established that the capillaries of the brain form an intricate network of anastomosing vessels, collaterals existing everywhere so that the capillaries are rarely, if ever, terminal. The blood has access consequently from any given arteriole, or arterioles, via these anastomoses to a number of efferent venules. As Lorente de Nó ('27) and Pfeifer have stressed, capillary anastomoses are so numerous that one can trace in injected preparations a continuous course via anastomoses from any point in the brain to any selected distant point. Large and small arteries and venules, moreover, do not, as a rule, accompany one another as homonymous pairs; instead, they follow separate courses in the great majority of instances, the capillary mesh occupying the intervening territory. Because of the ample anastomoses, involving capillaries and possibly small arteries and veins, end-arteries, in the sense of Cohnheim, have been questioned by Pfeifer ('28) and Cobb ('32) in the brain of cat, rabbit, monkey and man. These characteristics are accepted as applying to all mammalian brains thus far studied, and we can affirm the correctness of this account in injected material from cat, rat, guinea pig, rabbit, armadillo, rhesus monkey and man.

Much to one's surprise the pattern of the vessels supplying the brain tissue of the opossum is found to be totally different. It can be observed that each artery entering the brain from the pia receives at its point of entry a corresponding vein, a vena comitans (figs. 1 to 4). Thence forward in their subsequent branchings, arteries and anterioles are accompanied by homonymous veins and venules, so that an artery and vein are always paired. The capillaries arising from these paired vessels form, without exception, slender loops, the afferent ramus of the loop arising from an arteriole, while the efferent ramus of the loop invariably returns to the corresponding venule associated with the arteriole (figs. 1 to 4). From the surface of the brain inward a given artery and vein in their subsequent branching and rebranching down to and including the capillaries are a self-contained terminal unit, not anastomosing with other similar units. In consequence, it is impossible to pass by way of collateral channels, either large or small, into the system of another similar pair of arteries and veins. Indeed, if it were possible to pull an artery and vein with its branches and capillaries out of the brain substance, no tearing of any vessel, either large or small, would be necessary. The vascular units constitute complete end-systems down to and including their finest radicals, the capillaries, and are in consequence, the direct antithesis of the cerebral meshwork in other mammalian forms. We encounter the most complete system of end-vessels which it is possible to imagine. Not only is it a system of end-arteries in the original sense of Cohnheim, but even the capillaries end in separate non-anastomosing loops. In spite of being true end-systems, adjacent units overlap intricately and profusely by interdigitation of their respective capillary loops. Indeed, the hairpin-like terminal capillaries of several neighboring systems share the territory between them about

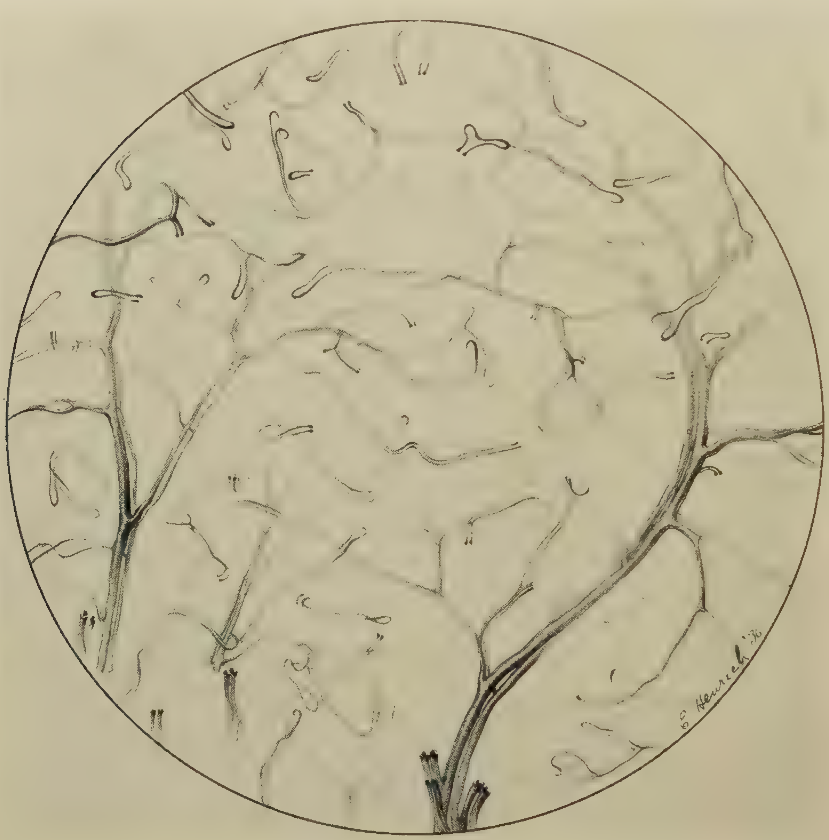


Fig. 1 Drawing showing the pattern of the blood vessels at the junction of the cerebral cortex and white matter. Some details are semidiagrammatic in this and the other drawings. × 94.

equally, thereby nourishing the brain tissue from independent sources. Because of the marked overlapping, occlusion of a vascular unit in the opossum's brain might not lead to complete infarction of the territory supplied by it. Another characteristic of the interdigitating loops, which twist like threads through the brain tissue, is that they avoid actual contact with one another and remain about equidistant in all places (figs. 1 and 8). Furthermore, the capillary loops avoid the larger vessels so completely that around each larger artery and its vena comitans one can see a distinct small avascular zone.

The vast majority of the capillaries form slender, hairpin-like loops. In each low power field, however, a few can be observed that, instead of ending in a simple loop, exhibit a staghorn-like termination, the terminal loop being bifid or having one longer and one lesser prong. Several of these rarer types of loops are illustrated in figure 6.

The vascular pattern described appears to be characteristic of the entire brain including forebrain, mid-brain, hind-brain, cerebellum and spinal cord. The arrangement of vessels applies to both gray and white matter alike, although, as one might anticipate, in the former the capillary loops are more numerous than in the latter.

A few areas require more specific mention. The optic nerves and retina exhibit the same capillary pattern as the rest of the brain, although in the optic trunks the loops are relatively few in number and rather coarse (fig. 10). Ordinary nerve trunks on the contrary—the maxillary devision of the trigeminal, for example—possess capillaries of the anastomosing type, instead of looped capillaries. Similarly, the dorsal and ventral roots of the spinal cord are supplied by twigs from the meninges which run longitudinally between the nerve bundles as single capillaries which now and then anastomose.

The pial blood vessels running in the pia-arachnoid and traversing the surface of the brain do not differ essentially, as far as we can ascertain, from the configuration seen in other mammalian brains. The pial vessels are not paired, and the major ones with their tributaries have similar topographical relations to those seen in other forms. The pairing of vessels, characteristic of the opossum brain, applies only to the vessels actually within the brain substance. A penetrating artery and an emerging vein, in partnership, can be traced to the brain surface, upon reaching which the two vessels can be seen to separate immediately, the artery arising from any one of several pial arteries in the neighborhood, the vein draining into a nearby venous channel (fig. 2). The parent

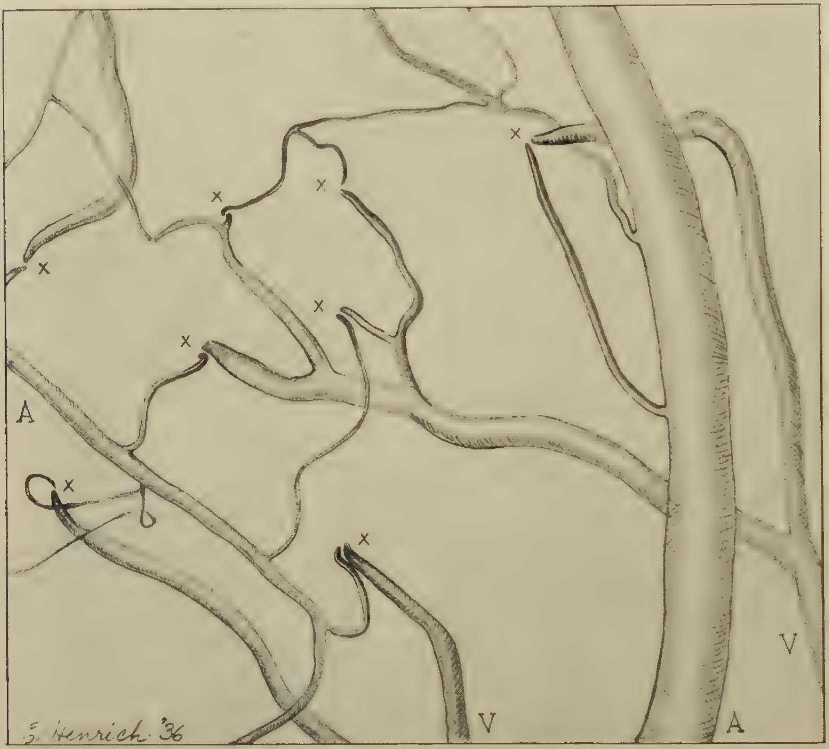


Fig. 2 A portion of the meninges from the cerebral hemispheres illustrating the manner in which the paired vessels penetrating the brain arise from the meningeal pial vessels. Arteries are marked A; veins, V. The small crosses indicate the pairing of a pial artery with a vein where they are about to penetrate the brain. Note how much larger the veins are than their corresponding arteries. × 94.

pial vessels, although of necessity located in the same vicinity, show not the least indication of being paired. Of the arteries and veins that penetrate the brain in pairs, the vein is, as a rule, at least twice as large as the corresponding artery (figs. 2 and 3).

The vessels of the choroid plexuses, unlike those penetrating the brain, are plexiform and show no tendency whatsoever to run in pairs (fig. 5), in this regard being identical with the choroid plexuses of other mammals.

The processus infundibuli of the hypophysis constitutes a small but interesting exception to the general vascular pattern described. The well-injected infundibular process displays within its substance arteries and veins which are not paired and which communicate, moreover, with a plexus of large capillaries which anastomose freely and exhibit no terminal capillary loops whatsoever. The infundibular stalk is also of some interest. It is ensheathed by a particularly rich network of pial vessels from which a few discrete capillary tufts of plexiform character penetrate the poorly vascularized interior of the stalk. The hypothalamus proper, including the tuber cinereum, is, on the other hand, abundantly vascularized by terminal capillary loops like the rest of the brain. At the junction of the tuber cinereum and stalk the terminal thread-like capillary loops cease for the most part. There are, however, perhaps a dozen terminal loops which appear to stray from the tuber cinereum for a short distance into the territory of the stalk. These terminate without anastomosing with one another; but they end, nevertheless, not as single thread-like loops, but each as a minute capillary plexus. Anastomoses between the capillaries of hypothalamus and stalk, which occur regularly in other mammals, here clearly do not exist.

The cerebellum shows interesting variations of the general pattern (fig. 3). Paired arteries and veins penetrate the cerebellar substance to the junction between molecular and granular layers. Here the vessels branch, giving rise, first, to smaller paired vessels and capillaries which reverse their course, extending outward toward the surface of the molecular layer and terminating at various levels in capillary loops. These loops in the molecular layer have a distinct tendency to be parallel to one another. The branching vessels at the junction of molecular and granular layers give rise, second, to slender paired vessels and capillary loops which penetrate the granular layer and the white matter beneath. The granular layer receives a rich supply of capillary loops, whereas the subjacent white matter is much less well supplied (fig. 3).

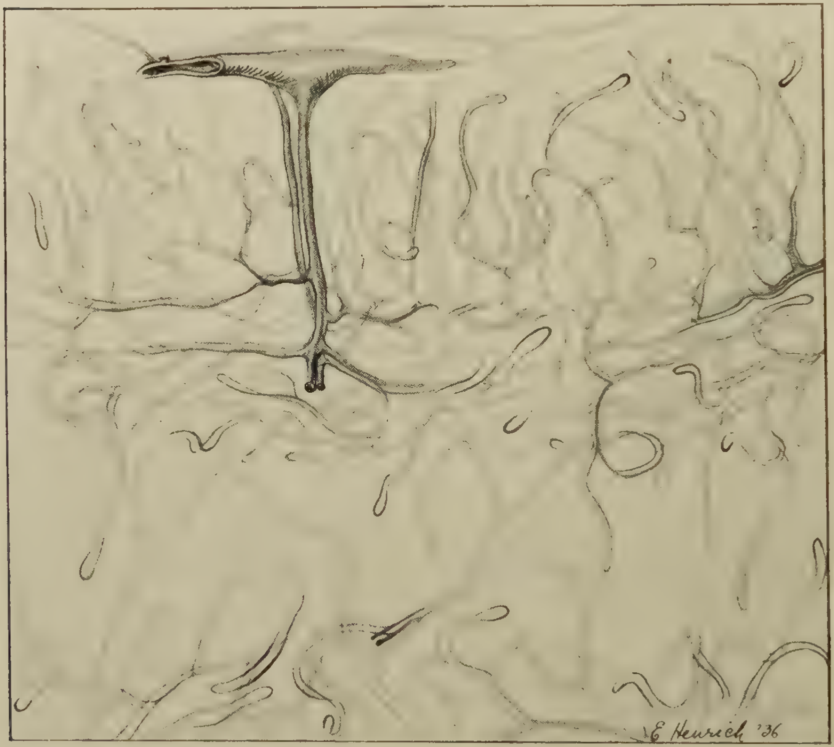


Fig. 3 Drawing showing the pattern of the vessels supplying the cerebellar layers, as well as the white matter. Note that the capillary loops in the molecular layer have a tendency to run perpendicularly to the surface, while those in the granular layer run in various directions. Note also that in the strip of white matter, below the center of the figure, the capillary loops are scarce. Of the artery and vein supplying this area from the pia, observe that the vein is much larger than the corresponding artery. × 140.

The spinal cord is supplied by successive paired vessels lying in the anterior median fissure which give off branches to the gray matter. These small branches break up into numerous short loops within the gray matter, while longer loops extend out into the white matter (fig. 4). The cord also receives paired vessels which enter the white matter directly from the periphery. The latter pass, as a rule, through the white matter to the border of the gray before breaking up into the smallest twigs from which terminal capillary loops either return to supply the white matter or extend centrally into the gray substance. As has been indicated, the gray

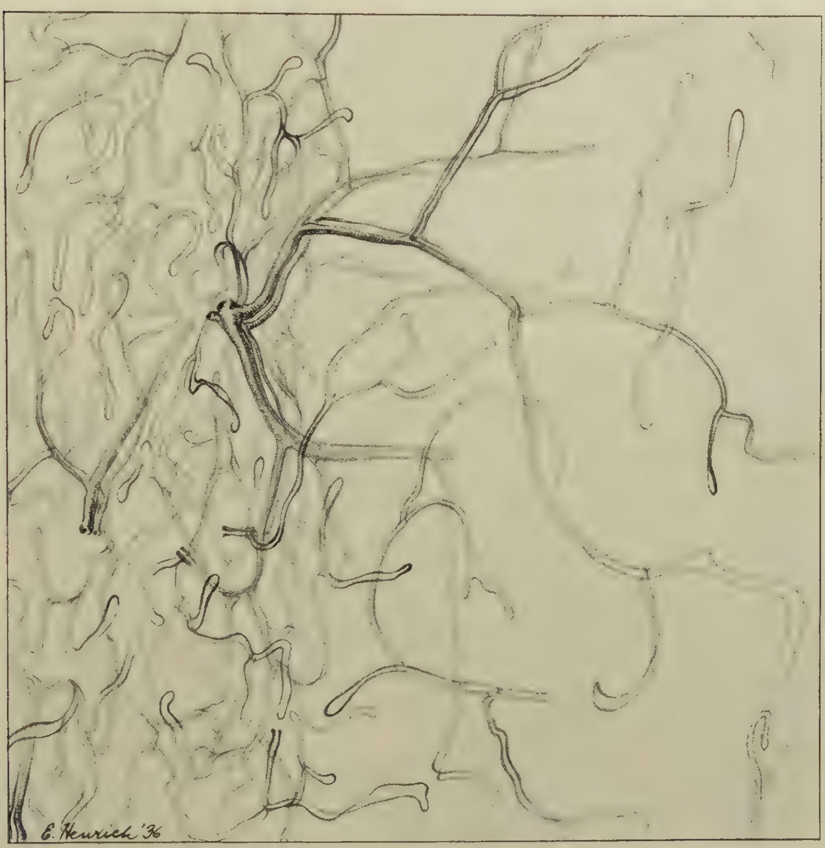
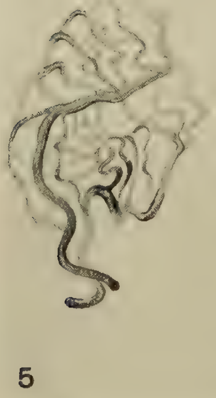


Fig. 4 Drawing of the blood vessels supplying the spinal cord viewed in a preparation cut longitudinally to include part of the white and gray matter. The gray matter situated on the left is more copiously supplied with capillary loops than the white matter on the right. × 140.

matter is much more richly supplied with capillary loops than the white. The capillary loops, whether they arise from the vessels entering by way of the median fissure or from the above-mentioned vessels which penetrate from the outside in, finally supply the white matter in a somewhat radial pattern passing for a variable distance from the center toward the periphery of the cord. The loops in general, consequently, do not run parallel to the fiber tracts in the cord. Interesting, however, is the observation that almost invariably the two threads or limbs composing an individual loop lie in a longitudinal plane projected through the cord and not in the transverse plane which would be the alternate possibility. As a result of this arrangement, if the white matter of the cord is cut lengthwise (fig. 4), the looped character of the capillaries is apparent at a glance; if, on the other hand, a transverse slab of cord is examined, the loops in the white matter appear for the most part as single threads, because the one thread lies concealed beneath the other. In this respect the capillaries are definitely oriented in reference to the intervening bundles of nerve fibers.



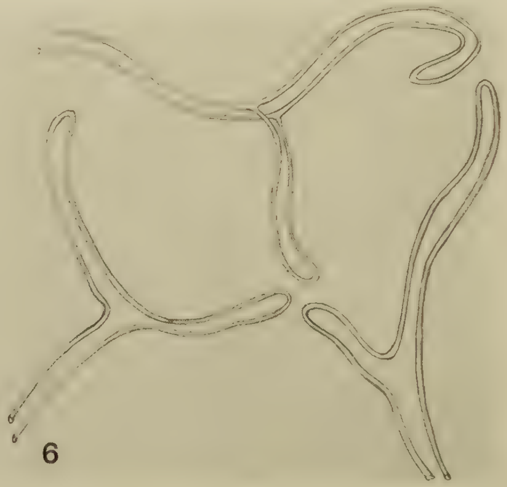


Fig. 5 A fragment of choroid plexus showing the meshwork formed by its vessels. × 140.

Fig. 6 Detailed sketches of individual loops showing some of the rarer forms.

DISCUSSION

We are not aware of the existence in any other mammal of a cerebral vascular pattern similar to the one described here in the opossum. Cat, rabbit, rat, guinea pig, armadillo, rhesus monkey and man can be ruled out through our own observations. From the careful study of Sterzi ('04) on the finer vessels of the spinal cord, one can also dismiss with some confidence the likelihood of the occurrence of the condition in Insectivora, Chiroptera or ungulates, for he studied the hedgehog, mole, several bats, the horse and cow. To our knowledge the finer vascular pattern has never been investigated, however, in other marsupials or in the monotremes, a step which would be desirable.

Turning to lower vertebrates, one discovers that the subject of capillary pattern in the central nervous system has been rather carefully studied by Schöbl (1878, 1882) and Sterzi ('04). Indeed Schöbl reports the discovery of a vascular pattern in the brains of many urodele amphibians and in the majority of lizards very similar to the condition described here in the opossum. Sterzi ('04) confirms in major particulars the discovery of Schöbl. In the urodele amphibians there are no actual arteries or veins entering the brain substance; instead, simple, long terminal capillary loops penetrate the neural tube from the larger vessels in the meninges. In the Lacertilia, on the other hand, the penetration of arteries and veins in pairs and the formation of terminal loops are almost identical with the pattern which we have observed in the opossum.

On the contrary, in all other lower vertebrates, namely, fishes, anuran amphibia, snakes, crocodiles, tortoises and in birds, Sterzi describes the anastomosing pattern of the cerebral capillaries as occurring without exception. For several fishes and a bird the observations of Craigie ('27 a and b, '28, '29) substantiate this conclusion. Moreover, in two frogs and two snakes (Natrix sipedon and Coluber constrictor), of which we have injected brains, the anastomosing pattern of the cerebral capillaries is amply demonstrable.

Among the Lacertilia, in which the condition of terminal looped capillaries generally prevails, both Schöbl and Sterzi find an exception in Chamaeleo vulgaris. Moreover, in Varanus arenarius and in Stellio vulgaris, Sterzi recognizes composite or transition forms between brains possessing terminal loops and those supplied typically with a capillary meshwork. Sterzi, moreover, claims that the pattern of terminal loops is primary or more primitive than the anastomosis of the capillaries to constitute a network. This belief, however, is open to question and deserves further examination on both embryological and phylogenetic grounds. Meanwhile, the facts at hand could be interpreted as indicating that the condition of terminal loops is a form of specialization of the capillary bed which has arisen independently in several widely separated groups of vertebrates.

SUMMARY

1. An unusual pattern of the blood vessels supplying the brain and cord of the opossum (Didelphys virginiana) has been found. The blood vessels enter the substance of the brain and cord in pairs, consisting of an artery and vein. These continue to branch in pairs down to, and including, the capillaries which consist of thread-like, terminal loops. Neither the paired vessels nor the terminal capillaries anastomose with other similar vessels. The brain is supplied consequently by distinct and separate vascular units which do not anastomose with one another. These units consist of end-arteries in the sense of Cohnheim with the additional feature that the ultimate capillaries are also end-vessels. Although the capillaries of adjacent systems do not unite, the capillary loops of contiguous units interdigitate freely.

2. The opossum is the only mammal so far examined in which a vascular pattern of this nature has been observed. In some insectivores, ungulates, rodents, carnivores and bats, besides in the armadillo, rhesus monkey and in man, the capillary bed is known to be of the network type. Other marsupials and monotremes have not been thus far examined.

3. The peculiar vascular pattern observed in the opossum brain has been described before in lizards. In some urodele amphibians, also, a similar, but much simpler pattern of capillary loops has been previously described. In fishes, anuran amphibia, snakes, tortoises, crocodiles and birds, on the other hand, only capillary networks have been observed.

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PLATE 1

EXPLANATION OF FIGURES

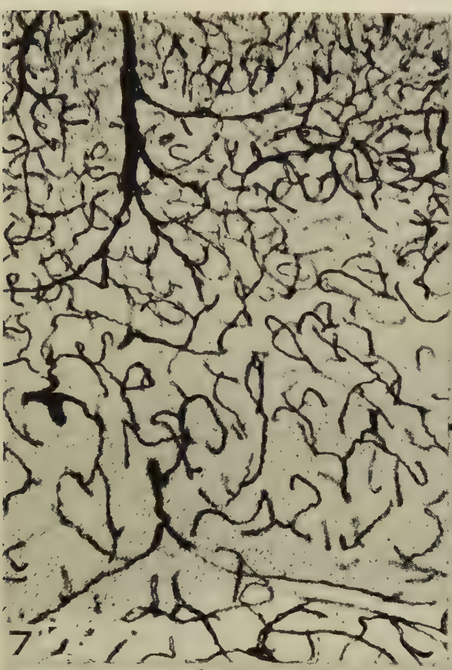
7 Photograph of the cerebral cortex showing blood vessels of gray and white matter. × 50.

8 Photograph of cerebral cortex showing individual capillary loops. × 115.

9 Photograph of border of cortex showing the area from which the semidiagrammatic drawing (fig. 1) was prepared. × 73.

10 Photograph of capillary loops in optic nerve. The loops are coarser here than in other parts of the brain. × 73.

11 Photograph of capillary loops as seen in a 20 μ section stained with haematoxylin and eosin. × 73.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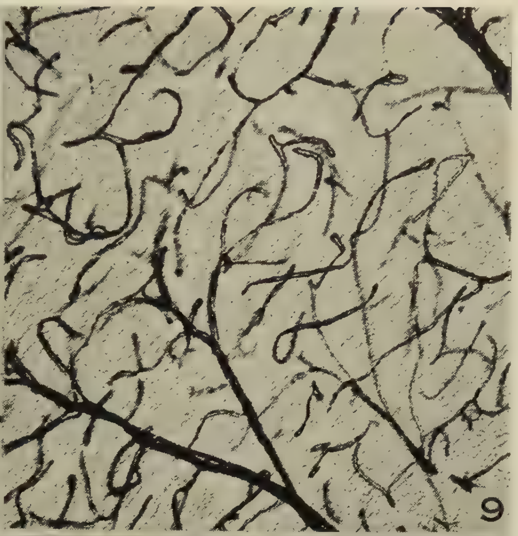
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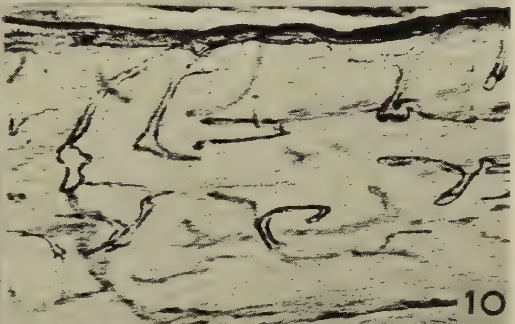
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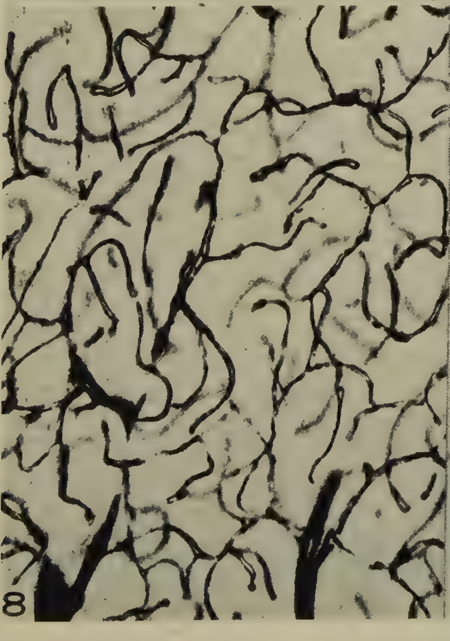
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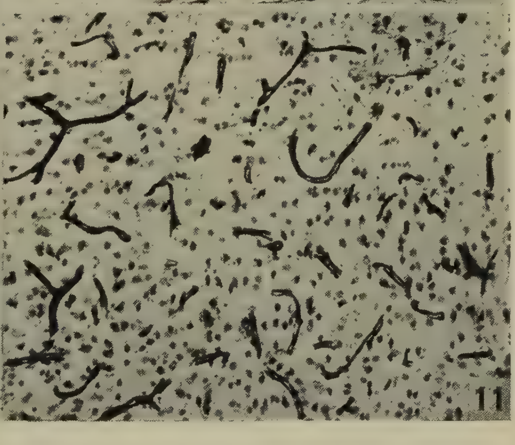
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THE PRIMARY TYPES OF EXTRA-ORGANIC GROSS CONNECTIVE TISSUE STRUCTURES

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Our understanding of the more detailed gross features of connective tissue is limited by the inadequacy of methods frequently employed. Descriptions of the technique of preparation tend to be vague. Methods for gaining a satisfactory three-dimensional view of the undisturbed tissue have only rarely been used. Another serious limitation is that the bodies examined have not been chosen to represent sufficiently restricted anatomical types. When these requirements will have been met, our conceptions of structural details of extra-organic gross connective tissue formations will require much revision and expansion. This need not discourage the attempt, however, at this time to develop a consistent classification and terminology.

The lack of a comprehensive survey of the elements of the subject is not due to an absence of sufficient information. We have, for instance, such earlier contributions as those of Roux, His, Eisler, Sterzi and others, dealing in a masterly way, from the viewpoint of the time in which they were published, with various extra-organic gross connective tissue formations and also a few recent studies utilizing more modern methods. The survey presented here has been subjected to criticism and checked by dissections in the anatomical course for several years. It has been paralleled by investigations, soon to be published, of certain extra-organic gross connective tissue structures.

Probably no other gross anatomical field causes the medical student, year after year, such confusion of mind. Not only do all textbooks of gross anatomy lack an introductory survey of extra-organic connective tissue formations, but apparently the special literature also offers no such discussion. No enumeration of the primary divisions was found in texts or other writings that can claim to be reasonably complete. Some primary divisions have not even been recognized by a name in descriptive normal anatomy.

As a preliminary to the discussion of the connective tissue types, certain definitions of microscopical terms and of the major bodily divisions must be recognized.

The view is here taken that with well-embalmed material, a gross anatomist can distinguish, without microscopical aid, five gradations in density of that fibrous connective tissue which is characterized especially by its content of collagenous and elastic fibers. These types are: 1) Very loose fibrous tissue, also termed rarefied areolar tissue; 2) ordinary loose fibrous tissue, or areolar tissue; 3) fibrous tissue of intermediate density; 4) dense fibrous tissue in which the collagenous fibers tend toward a parallel arrangement, forming a fairly dense, felty mass; and, finally, 5) very dense fibrous tissue, in which most of the collagenous fibers are closely packed together into one or more systems of parallel fibers, forming bodies with mirror-like surfaces. The application of this classification of connective tissue to extra-organic formations can be, of course, only approximate in many regions because of intergradations and the frequent changing of the connective tissue picture even within small areas.

There are two generally recognized modes of dividing the body into primary parts. One may be termed the linear and the other the circumferential. The linear plan, which recognizes such divisions as head, neck, thorax and the like, enters but little into a discussion of the major types of extra-organic gross connective tissue structures, but is used rather for designating subdivisions of these types. The circumferential plan is more fundamental for the consideration of extra-organic gross connective tissue structures and is necessary for a definition of their primary types. According to it, we have four bodily divisions: 1) The cutaneo-subcutaneous; 2) the musculo-skeletal; 3) the central nervous; 4) the sero-subserous or parieto-visceral, including, for example, the extra-organic connective tissue of the mediastina. The third, termed central nervous, is embedded in the musculo-skeletal layer and comprises the cord, brain, optic nerves with their membranes and a part of the eyeball.

Ten primary groups of extra-organic gross connective tissue structures will be recognized in this study. They may be briefly characterized as follows:

1. *Panniculus adiposus,* deposits of fatty areolar tissue either alone or taken with a supporting fibrous alveolar formation making a coarser sponge work than that of the fibrous element of the fatty tissue itself.

2. *Shearing layers,* most characteristically formed of very loose fibrous tissue and always lying between two surfaces that glide on one another.

3. *Organ sheaths,* fibrous sheets paralleling the surfaces of organs or groups of organs and separated from them by a fluid-filled space or by a shearing layer. Sheets of this nature investing tendons are excluded from the group.

4. *Retinacula,* usually fibrous sheets separated from the surfaces of tendons either by a fluid filled space or by a shearing layer. A few have the form of rings. Retinacula hold tendons down to bones.

5. *a. Distant fasciae,* fibrous sheets paralleling the surfaces of organs or groups of organs and separated from them by a considerable thickness of connective tissue which is usually panniculus adiposus.

b. Contiguous fasciae, fibrous sheets adherent to the connective tissue capsule of organs or groups of organs.

6. *Retaining tracts,* serving organs of three body divisions by holding them to the great supporting component of the body the musculo-skeletal layer either for their support or to limit such of their movements as are not due to gravity.

7. Mesenterial layers whose chief functions are the limitation of the excursions of certain highly mobile viscera and the transmission of vessels and nerves to them.

8. Ligaments, holding skeletal elements together, or occasionally connecting two parts of a single skeletal element.

9. Remnants, those connective tissue vestiges of vanished organs or parts of organs, which have not a structure and function admitting them to one of the foregoing groups.

10. Blends, combinations of one or more of the other groups with one or more intra-organic connective tissue structures such as aponeuroses, joint capsules, the capsule of a bone and perhaps the capsules of muscles and glands.

PANNICULUS ADIPOSUS

Deposits of fatty areolar tissue, taken with the coarser fibrous sponge which often permeates them, have long been termed panniculi adiposi when occurring in the cutaneo-subcutaneous layer. Because a name is lacking for gross deposits of this nature which is accepted for all parts of the body, the term panniculus adiposus will here be extended to extra-organic gross fatty areolar formations with any associated coarser connective tissue sponge-work, in all of the four circumferential layers of the body. The term, 'panniculus,' meaning in Latin, a small piece of cloth, describes adequately the laminar form of most fatty areolar deposits, but it must be granted that some of them, as, for example, many periarticular fat masses, assume a variety of other shapes.

SHEARING LAYERS

Rarefied areolar tissue, and perhaps at times, typical areolar tissue, occurs in layers in many parts of the body as one of the most structurally and functionally clean-cut primary divisions of gross connective tissue formations. Probably the reason it has not been recognized before as an entity in gross anatomy is the dominating interest in its pathological condition, when (due to its disruption by accumulating pathological fluid) it has been replaced by a fluid-filled space. Its rarefied areolar tissue readily yields to the hands of the dissector by gaping open to form cavities filled with air. Again cavities may be formed in it for purposes of anatomical study by the injection of fluids, as, for example, warm gelatin. Among the shearing layers are the strata of very loose connective tissue between the sheath of the biceps humeri and its epimysium, the layer between the fascia of the subscapularis and the serratus anterior, the layer between the sheath of the submandibular gland and its capsule and the delicate tissue which forms the sub-fascial or so-called Tenon's 'space' surrounding part of the eyeball.

As shearing layers lie between the surfaces of two organs or structures that frequently slip on one another, they are subjected predominantly to a shearing action, and so must be of a very yielding texture. Their fibers therefore are loosely aggregated and there is much ground substance.

Most of the larger shearing layers are in the musculo-skeletal division of the body. Information is very incomplete about the shearing layers related to the surfaces of small structures.

INVESTING FIBROUS SHEETS TERMED SHEATHS, RETINACULA AND FASCIAE

Four types of fibrous sheets paralleling more or less the surface of an organ or group of organs are familiar to anatomists. These are here termed sheaths, retinacula and two varieties of fasciae, the distant and the contiguous. These formations seem, in most cases, to be adapted primarily to react to forces emanating from some organ or group of organs whose surface they parallel. Also in the case of fasciae, organs which may attach to their edges seem, frequently, by their movements to exert an important mechanical influence upon them. These various formations vary as to the intimacy of their relationship to the organs they parallel and as to the amount of movement between the two.

Sheaths usually consist of dense, very dense, or intermediate fibrous tissue. They are characterized by a considerable degree of mobility relative to their organs. Two types of sheaths may be distinguished depending on the nature of the intervening tissue and the degree of freedom of movement. The more mobile type is separated from the organ by a fluid-filled space. An example of this type is the fibrous layer of the parietal pericardium. The less mobile variety is separated from its organ by a shearing layer. An illustration is the sheath of the submandibular gland.

Sheaths are present in all the four major subdivisions of the body. There seems little definite information as to their presence around smaller structures.

Retinacula. The term is here applied in accordance with the suggestion of the German committee on terminology to the fibrous sheets and occasional rings which hold tendons down to bones. There is a marked gliding of the tendons on their retinacula. They are usually formed of very dense or dense fibrous tissue. Frequently the retinaculum is separated from the tendon by a cavity which with its more or less complete mesothelial lining and its mucoid content constitutes the sheath bursa of the Basle nomenclature. It would be more consistent to name these structures retinacular mucous bursae. Examples of this variety of retinacula are the fibrous digital sheaths of the Basle nomenclature. Other retinacula are separated from their tendons by shearing layers. The transverse crural 'ligament' falls within this group. Retinacula are found only in the musculoskeletal layer.

Fasciae consist usually of dense or intermediate collagenous-elastic connective tissue. In a few instances, parts of these sheets assume the very dense form. The displacements of the organs relative to the fasciae seem on the whole less than that observed for sheaths. Fasciae include two sub-types differing somewhat in topography and function. That which we will term distant fascia is usually separated from the organ it parallels by a panniculus adiposus and shows the greater motion relative to its organ. The renal and Scarpa's fasciae are examples. The other type is here termed contiguous fascia because it is in contact with the capsule of its organ. Examples of contiguous fascia are the fibrous layers investing the superficial surface of the parotid gland and of the pectoralis major muscle.

Contiguous fasciae are found chiefly in the musculo-skeletal layer. Distant fasciae are especially characteristic of the cutaneo-subcutaneous and the sero-subserous layers. Distant fasciae sometimes indicate a second important mechanical activity by attaching at their borders to mobile organs. The structural and functional differences existing between contiguous and distant fasciae are of sufficient importance to raise a doubt as to the propriety of combining them to form a single primary group.

The subdivisions of the group fasciae usually accepted are distinguished by considerations of topography. The designations applied to some of them will be discussed because they are inappropriate and cause the medical student much difficulty. The muscular fasciae are referred to as 'deep' fasciae, whereas logically it is the subserous or parieto-visceral fasciae which merit the term deep. The muscular fasciae are in reality intermediate in position between the fasciae of the cutaneo-subcutaneous and the sero-subserous layers. The terms subcutaneous (O.T. superficial), muscular (O.T. deep) and subserous or parieto-visceral are more suitable than the terms superficial, intermediate and deep, because more descriptive. They will be adopted here.

Probably the greatest confusion in the terminology of extra-organic gross connective tissue structures experienced by the medical student is encountered when he desires to contrast the muscular and the subserous fasciae. The term endothoracic is usually applied to the internal muscular fascia of the thoracic wall, or, in other words, of the musculo-skeletal layer, and the designation endo-abdominal is accepted by many for the corresponding layer lining the abdominal wall, cranial to the linea terminalis. Yet these fasciae are not within the body wall as the prefix 'endo' would indicate, but are themselves part of the wall. The expression fascia pelvis of the Basle nomenclature for the internal muscular fascia of the pelvis is also unreasonable and confusing because this designation obviously should apply to the total range of fasciae of the pelvis, rather than to one subdivision of it. The term endopelvic for the fasciae in the subserous connective tissue layer in the pelvis is not a misnomer nor would the terms endothoracic and endoabdominal be unreasonable if used similarly in thorax and abdomen. Subserous pelvic, subserous thoracic and subserous abdominal seem much more appropriate designations, however, for these fasciae because these expressions are a part of our consistent scheme of terminology and are more precise and more descriptive.

The application of the term subserous to fasciae of the deepest circumferential layer is desirable as paralleling the designation subcutaneous but it cannot well be used in the head and neck. This is because of the withdrawal of the coelom and its serous lining from these regions during the course of development. In the head and neck, therefore, we must have recourse to the adjective visceral. We are here using the term visceral in its gross anatomical rather than in its neurological sense. In the neck a part of the prevertebral and of the second layer of the muscular fascia (infrahyoid layer) rank as internal muscular fasciae.

RETAINING TRACTS

The presence of these tracts is explained by a fundamental feature of bodily architecture. The cylindrical musculo-skeletal layer, together with its projection into the extremities, plays the chief part in supporting the weight of the body. It is to be anticipated therefore that various organs in the cutaneo-subcutaneous layer, the sero-subserous layer and the central neural division should be bound to the musculo-skeletal by fibrous condensations which in certain postures transmit more or less of their weight to this layer. These condensations are here given the name retaining tracts although it should, of course, be recognized that all types of extra-organic gross connective tissue structures serve in greater or lesser degree to retain organs in their positions. Examples of retaining tracts are the 'retinaculae' cutis and the pubo-vesicle 'ligament.' The connective tissue formations connecting the pia of the cord to the vertebral periosteum taken together constitute a retaining tract.

Another less important function of probably all retaining tracts is the limitation of some movements of organs not due to the pull of gravity.

Retaining tracts are made up of intermediate or dense and perhaps at times of very dense fibrous connective tissue.

MESENTERIAL LAYERS

These are the proper connective tissue laminae of mesenteries. The expression also is taken as including those semifixed sheets called 'fasciae of fusion' and the non-peritoneal connective tissue element of those so-called ligaments passing from one abdominal viscus to another, such as the lienogastric ligament derived from the original mesenterium commune. A function of mesenterial layers is the limitation of the normal excursions of a viscus. They frequently also transmit vessels and nerves to it. They are closely related to retaining tracts in function. The connective tissue of which they are formed seems usually to be of the intermediate grade. Their rather weak structure is in harmony with the moderate character of the mechanical demands placed upon them.

LIGAMENTS

The exclusion of tracts binding organs to the musculoskeletal layer from the connective tissue group termed ligaments leaves this designation free for its primary, sharply defined, and long accepted meaning, namely, that of a connective tissue structure holding skeletal elements together. Occasionally a ligament connects two parts of a single skeletal element, as that between the coracoid and acromial processes of the scapula. In the interests of clear thinking, formations fused to joint capsules should be included among the ligaments only provided they stand out with a certain degree of independence from the capsules. As joints are organs, their proper capsules are intra-organic gross connective tissue structures. When a capsule is partly fused to a ligament the structure as a whole becomes an example of our tenth type which it termed a blend.

REMNANTS

It is well known that connective tissue may replace an organ or part of an organ which existed at some time in the ancestral adult. Thus the muscular fascia intervening between the omo-hyoid and the sterno-hyoid muscles has probably replaced a part of the group of infra-hyoid muscles. Connective tissue formations which replace organs or parts of organs which served to relate the individual to the mother before birth also should be considered here such as for example the round ligament of the liver. It will be granted that many connective tissue residues or organs are of a nature or are reconstructed to take on a form and function that warrants their inclusion in one of the other primary groups already discussed. The term remnant is not here defined to include such residues, but is applied only to formations that do not clearly fall within any of the preceding groups. The group is a provisional one as it would seem difficult to establish the right of specific connective tissue structures to be placed within it.

BLENDS

The term, blend, is here chosen for connective tissue structures which form an intimately related whole, and yet can be resolved functionally and often, in part, structurally into different primary gross connective tissue types. Apparently all blends include at least one extra-organic and one intra-organic connective tissue type. Thus, the interosseous membrane of the antibrachium contains as an intra-organic element, tendinous fibers of origin of the flexor digitorum profundus, and as an extra-organic constituent, fibers of ligamentous function passing between the radius and ulna.

We have found three groups of blends in the body: 1) Combinations of aponeuroses with fasciae or ligaments, as illustrated by the foregoing example. This is the most frequent type. 2) More or less complete fusion of ligaments to the proper connective tissue capsules of joints as the tibial collateral ligament and proper capsule of the knee joint, and 3) a single illustration of the fusion of one extra-organic type with a periosteum, namely, the cranial dura.

Perhaps further study will show that contiguous fasciae are at times so intimately related to the capsules of muscles and glands as to warrant grouping the two layers together as a blend. Other blends are the anterior crural, the lumbo-dorsal and the temporal so-called 'fasciae.' The larger intermuscular septae are usually, and interosseous membranes are always, blends.

THE ALLEGED TRANSMIGRATION OF THE LONG TENDON OF THE BICEPS BRACHII

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TEN FIGURES

It is generally accepted that the tendon of the long head of the biceps brachii (caput longum BNA), referred to as the long tendon in this article, develops outside the capsule of the shoulder joint but migrates through the capsule during later fetal development. Bardeen ('33) for example said that "the long tendon of the biceps lies primitively outside the capsule of the shoulder joint, but in some of the higher mammals has come to lie within the capsule." A similar point of view was expressed by Dwight ('30), Hepburn ('31), Chiarugi ('26), Testut ('11), Fick ('04), Stolowsky (1899), Le Double (1897) and Macalister (1889).

Most, if not all of these incidental and textbook statements seem to have been based upon the original observations of Hermann Welcker (1878), who examined fetal and adult stages of this tendon in a number of species. In some of these species he found it outside of the capsule of the adult shoulder joint. In others it was closely attached to the under surface of the capsule, and in still others it lay free in the articular cavity. However, his work on the human fetus was limited to what is discussed in the extract given below.

Since Welcker has been cited as authority for the belief that the long tendon of the biceps develops outside the shoulder joint and passes through the capsule during fetal development in man, it seems advisable to quote him verbatim. He wrote:

> Nach diesen Befunden würde kaum ein Zweifel bleiben, dass auch bei dem Menschen dieselbe Entwicklung stattfindet; doch gelang es mir, dies direct nachzuweisen. Bei einem menschlichen Embryo von etwa 3 Monaten (Länge vom Scheitel zum Steisse 62mm, Länge des Armes von der Schulter bis zu den Fingerspitzen 33mm) fand ich den intracapsulären Theil der Bicepssehne in einer Falte der Synovialhaut liegen (vergl. Fig. 4a). Das Gelenk wurde von hinten geöffnet und mit der Scheere quer durchschnitten, und der untere Abschnitt der Kapsel unter der Loupe untersucht. Bei Manipulirung mit der Nadel sieht man, dass die zur Sehne tretende Synovialhautduplicatur nahezu auf die Mitte der Sehne trifft; das Mesotenontium ist ziemlich knapp und noch etwas breit. Dasselbe lässt sich mittels der Nadel beliebig spannen, man sieht deutlich die von beiden Seiten von der Sehne zur Kapsel tretenden Synovialhautfalten.
>
> Bei einem zweiten menschlichen Embryo (Rumpflänge 72mm, Armlänge 37mm) war der Befund (Fig. 4b) wesentlich derselbe, doch fand sich die Unterminirung der Sehne hier fast einzig längs ihrer lateralen Seite.
>
> Ein dritter Embryo (Rumpflänge 82mm) zeigte die Bicepssehne bereits rings umgreifbar.

(The use of the word 'doch' in the first sentence is perplexing but I can offer no explanation for it.)

It should be noted that Welcker did not say that he saw the long tendon of the biceps outside of the capsule of the shoulder joint in the human fetus but concluded that in the human fetus, the tendon migrates through the capsule, apparently because he saw the former attached to the under surface of the latter by a fold of synovial membrane in a 62-mm. (V.B.) and a 72-mm. (V.B.) fetus, and also because he had found it in various positions in different species. That Welcker did not adequately demonstrate that the tendon migrates through the capsule of the shoulder joint is not stated by those who cited his work. In Keibel and Mall's Manual of Human Embryology, for example, Bardeen ('10) wrote:

> During fetal development the tendon of the long head of the biceps sinks in through the capsule of the joint. For a time it is covered by a layer of synovial membrane which attached it to the capsule, but in the third or fourth month it becomes free in the joint cavity (Welcker, 1878).

As far as I know, there are no reports in the literature giving the relationships of the long tendon of the biceps while it is outside the capsule of the shoulder joint or discussing the process by which it penetrates the joint capsule. This investigation was undertaken, at the suggestion of Doctor Meyer, to determine the position of the long tendon in the human fetus particularly in relation to the capsule of the shoulder joint in different stages of fetal development, and to determine by what process it obtains the intracapsular position.

For this investigation human fetuses and embryos preserved in formalin and varying in Vertex-Breech length from 13 to 66 mm. were used. In the larger fetuses blocks of tissues were obtained as follows. With the aid of a dissecting microscope the skin and subcutaneous tissues were removed from the upper one-half of the arm and from the shoulder region by the use of forceps and a dissecting needle. The attachment of the deltoid muscle to the humerus was cut and the muscle reflected medially to expose the capsule of the shoulder joint and enable me to identify the biceps muscle. This was then cut in its upper third and the proximal portion freed from the surrounding connective tissue. The attachment of the tendon of the short head to the muscle was also cut, leaving a small amount of the muscle attached to the long tendon. The latter was included in the block of tissue which was removed for purposes of identification of the long tendon in the sections made for microscopic study. A portion of the vault of the capsule of the shoulder joint, several millimeters in width to either side of the tendon, was removed from the rest of the capsule with a sharp knife. In order that the relationships of the long tendon to the internal surface of the capsule might not be disturbed, the cuts through the capsule were carried into the cartilaginous head of the humerus. The block of tissue removed hence was composed of a portion of cartilage from the head of the humerus and the overlying capsule of the shoulder joint with the long tendon. This material was embedded in paraffin and cut into serial sections 12 μ thick.

In the smaller fetuses, another method of obtaining blocks of tissue for sectioning was used, in order to avoid disturbing the relationships of the long tendon and the capsule. In these specimens the shoulder girdle was removed from the trunk by cutting the muscular attachments and the clavicle, and the brachium was divided transversely in its lower third. By this means, a block of tissue was obtained in which the shoulder joint and its surrounding structures lay undisturbed. The proximal two-thirds of the brachium were included in order that the long tendon could be identified positively in serial sections by following it to its attachment to the biceps muscle. This tissue was embedded in paraffin and cut into serial sections 12 μ thick. The two smallest embryos were embedded in their entirety and serially sectioned. All of the tissues were stained with Delafield's hematoxylin and counterstained with alcoholic eosin.

From the large number of sections studied, those shown in the photographs (figs. 1 to 10) were selected as best illustrating the relationships of the long tendon to the capsule of the shoulder joint during the pertinent period of the embryonic and fetal development of these structures.

For the purposes of this investigation, fetuses older than the one shown in figure 2 were felt to be unnecessary, for the only change from this to adult state is an increase in size of all the structures through growth and a complete disappearance of the fold of synovial membrane, the so-called mesotenontium of Welcker,[1] extending from the upper surface of the tendon to the under surface of the overlying capsule. In fetuses younger than the one from the sections for figures 9 and 10 were obtained, the joint capsule had not formed and the long tendon could not be identified.

Figure 2 is a frontal section through the shoulder joint of a 66-mm. fetus. The long tendon (b) is seen lying over the head of the humerus, just distal to its insertion into the supraglenoid lip of the scapula. The capsule of the joint is

[1] Listed in dictionaries as the mesotenonium, mesotendineum, mesotenon and mesotendon.

well developed and the tendon lies intra-articularly but is not free within the articular cavity, being attached to the under surface of the capsule by a fold of synovial membrane.

Figure 3, which is in the same plane of section as figure 2, shows the relationships present in a 51-mm. fetus. There is a well-developed joint capsule and the long tendon is intra-articular. A comparison of the two figures shows that in figure 2 many of the fibers making up the mesotenonium have disappeared, but they are clearly seen in figure 3 extending from the upper surface of the tendon to the under surface of the overlying capsule.

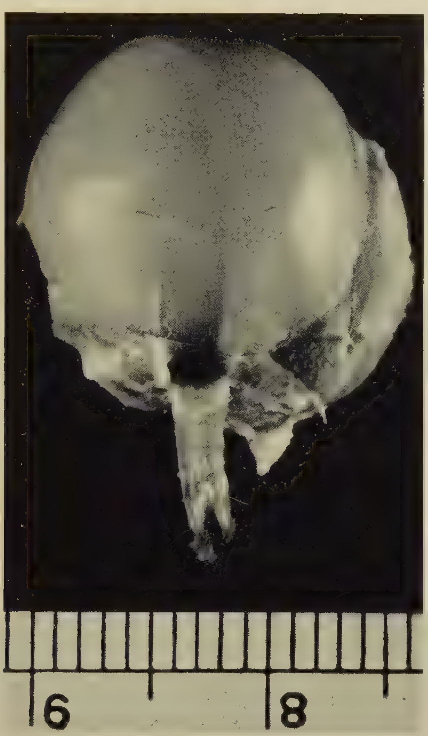

Fig. 1 Photograph of the head of the left humerus of a full term infant. Note the sulcus in the cartilage of the head of the humerus. The long tendon of the biceps is seen in the lower part of the figure and has been reflected to reveal the sulcus under it which may, in part, be a post-mortem impression. × 3.

In the transverse section through the shoulder joint of a 45-mm. fetus (fig. 4), the well-developed articular capsule is attached to the scapula and surrounds the outer surface of the head of the humerus. The long tendon lies against the internal surface of the capsule but is not free in the articular cavity. It is circumscribed and surrounded by a free zone except on its upper surface where the synovial attachment to the capsule is situated. The fold of synovial membrane surrounding the tendon and which separates it from the articular cavity is indicated by a thin layer below the tendon in figure 4.

The long tendon of a somewhat younger fetus is shown in oblique section at its insertion into the supraglenoid lip of the scapula in figure 5. This frontal section from a 41-mm. fetus shows the tendon below the capsule but still attached to it by a fold of synovial membrane, as in the previous specimen. The capsule is well-defined and there are no gaps in the continuity of the connective tissue fibers composing it. The tendon also is a well-defined structure and easily identified. The fragments seen in the articular cavity are pieces of cartilage detached from the humerus and scapula during preparation.

Figure 6 is a photograph of a transverse section from a 35-mm. fetus at a point above the bicipital groove and shows the tendon lying over the head of the humerus. There is a deep sulcus in the cartilage of the head of the humerus underneath the tendon, which lies beneath the capsule. Some

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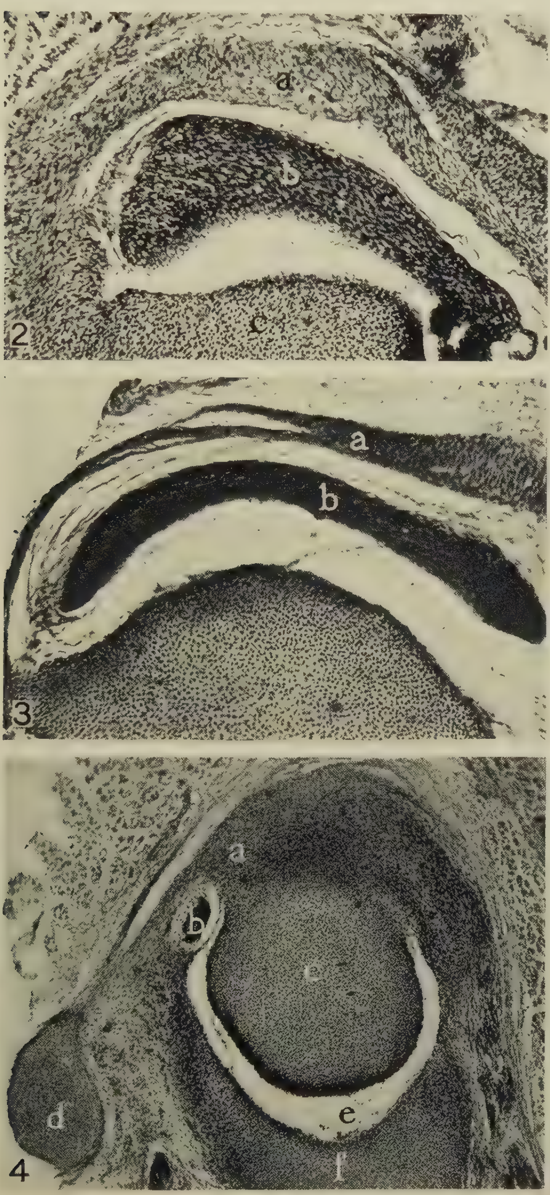
Fig. 2 Photograph of an approximately frontal section of the right shoulder of a 66-mm. (V.B.) fetus: a, the joint capsule; b, the long tendon of the biceps; c, the head of the humerus. × 100.

Fig. 3 Photograph of an approximately frontal section of the right shoulder of a 51-mm. (V.B.) fetus: a, the joint capsule; b, the long tendon of the biceps; c, the head of the humerus. Running from the tendon to the under surface of the capsule are the fibers which form the fold of synovial membrane or 'mesotenontium' of Welcker. × 100.

Fig. 4 Photograph of a transverse section of the left shoulder of a 45-mm. (V.B.) fetus: a, the joint capsule; b, a cross section of the long tendon of the biceps; c, the head of the humerus; d, the tip of the coracoid process; e, the joint cavity; f, the scapula. Note the synovial membrane holding the tendon to the under surface of the capsule. × 50.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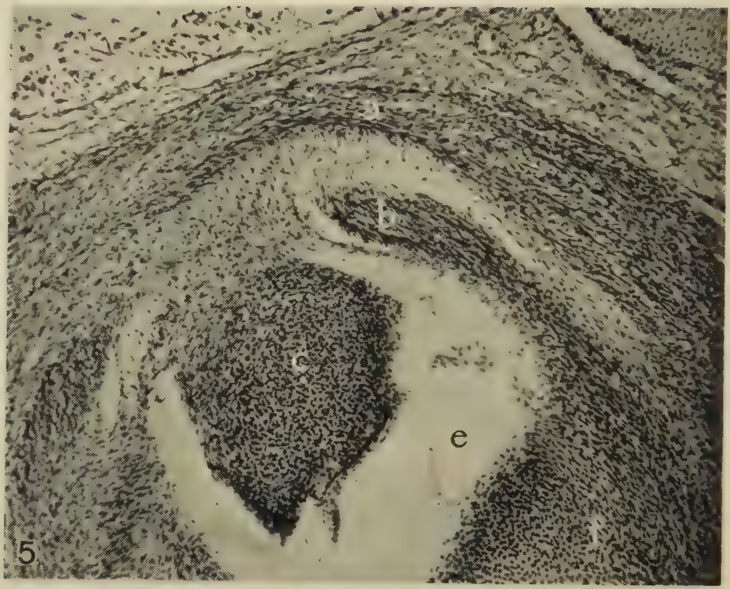
Figures 2 to 4



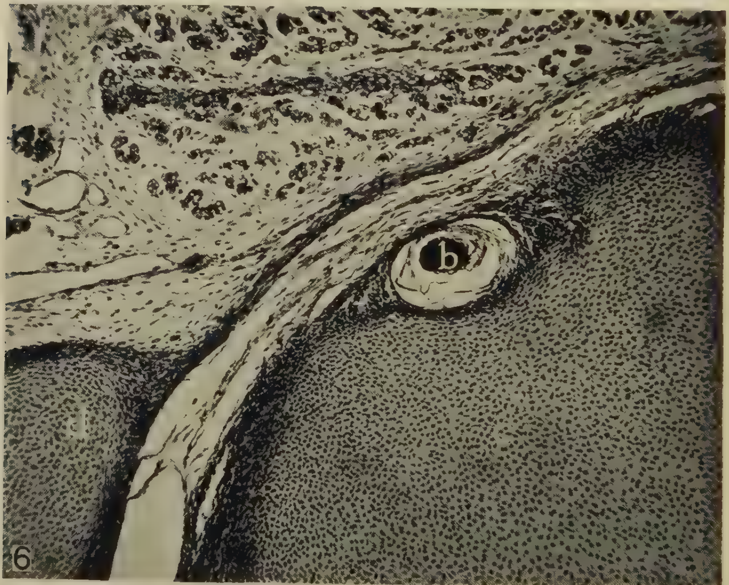


Fig. 5 Photograph of an approximately frontal section of the right shoulder of a 41-mm. (V.B.) fetus: a, the joint capsule; b, the long tendon of the biceps at its insertion into the supraglenoid lip of the scapula; c, the head of the humerus; e, the joint cavity; f, the scapula. × 125.

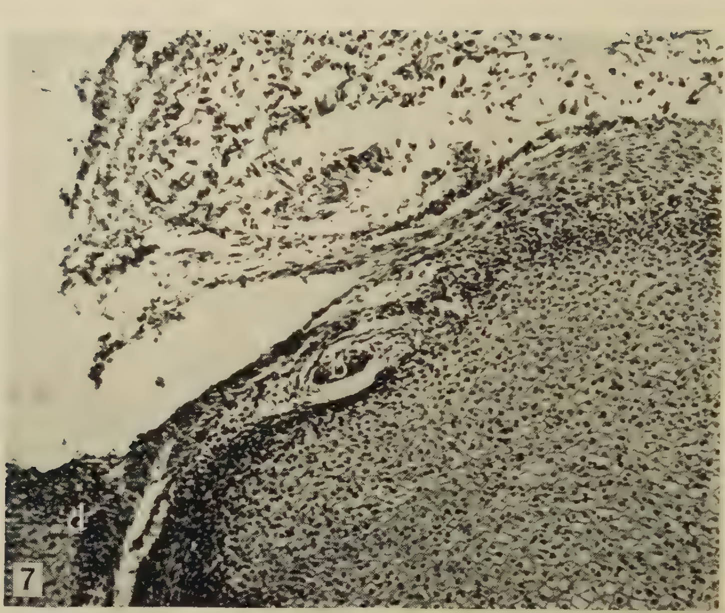
Fig. 6 Photograph of a transverse section of the right shoulder of a 35-mm. (V.B.) fetus: a, the joint capsule; b, a cross section of the long tendon of the biceps; c, the head of the humerus; d, the coracoid process. ×125.

fibers of connective tissue surround the tendon and attach it to the under surface of the latter. These fibers are not closely applied to the tendon but are loosely arranged about it, leaving a free space between it and the head of the humerus.

The occurrence of a sulcus in the cartilage of the head of the humerus in a fetus is surprising. I understand that when observed in the adult, such sulci have been attributed to wear from or impressions of the long tendon over the head of the humerus, but such an explanation is doubtful for it cannot account for a sulcus such as seen in figure 6. This could be regarded as resulting from interference with the growth of the cartilage by the developing long tendon; and if sulci found in the articular cartilage of the humeral head, near the tuberosities, are the result of developmental factors, one should expect them to be present more frequently in the infant than in the adult. To obtain evidence on this question, fifty humeri of infants at term and fifty-five humeri of adults from the dissecting room were examined. Among the fifty humeri of infants, thirty-six males and fourteen females, there were thirteen (26%) in which sulci in the cartilage of the head of the humerus such as represented in figure 1 were observed. They were bilateral in ten instances, dextral alone in two and sinistral alone in one instance. Twelve sulci were found in male specimens and one in a female. They were from $\frac{1}{3}$ to $\frac{1}{2}$ mm. deep, from 2 to 3 mm. wide, and from 9 to 12 mm. long.

Among the fifty-five adult humeri there was only one instance of a sulcus in the cartilage, continuing the intertubercular sulcus on to the head of the humerus. This was on the left side in a male cadaver. The sulcus was about $\frac{1}{3}$ mm. deep, about 6 mm. wide and about 30 mm. long and hence may in part at least be a post-mortem impression.

Figure 7 shows that the relationships of the long tendon to the joint capsule are the same in the 30-mm. as in the 35-mm. fetus (fig. 6), except that the fibers of the tendon are fewer in number and are not so densely packed together and that there are fewer fibers composing the joint capsule in the former.



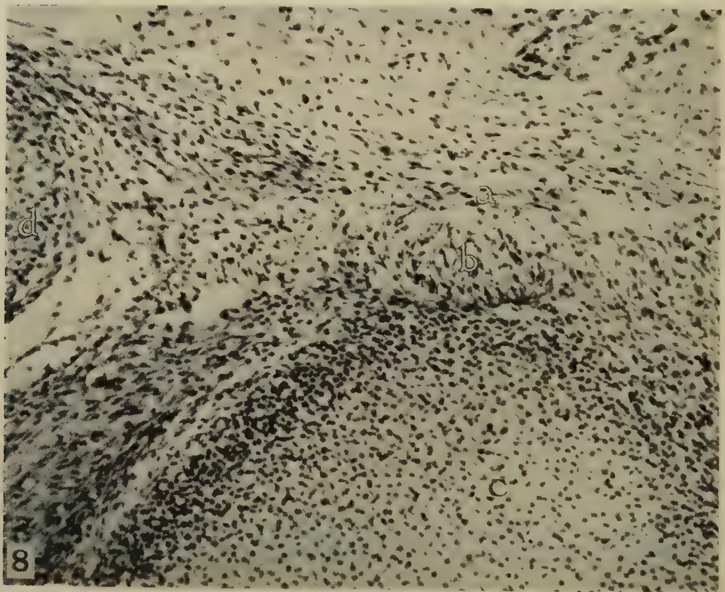
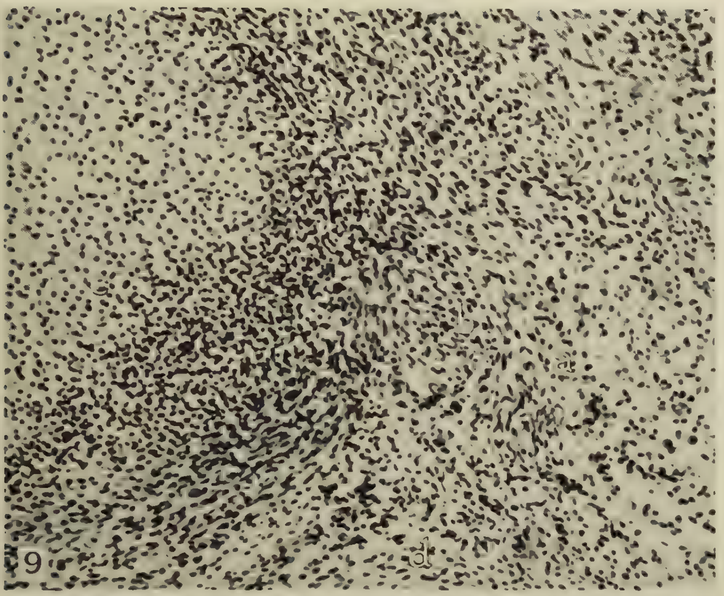


Fig. 7 Photograph of a transverse section of the left shoulder of a 30-mm. (V.B.) fetus: a, the joint capsule; b, a cross section of the long tendon of the biceps; c, the head of the humerus; d, the coracoid process. × 125.

Fig. 8 Photograph of a transverse section of the right shoulder of a 28-mm. (V.B.) fetus: a, the fibers of connective tissue which represent the joint capsule at this stage; b, a cross section of the long tendon of the biceps; c, the head of the humerus; d, the coracoid process. × 225.



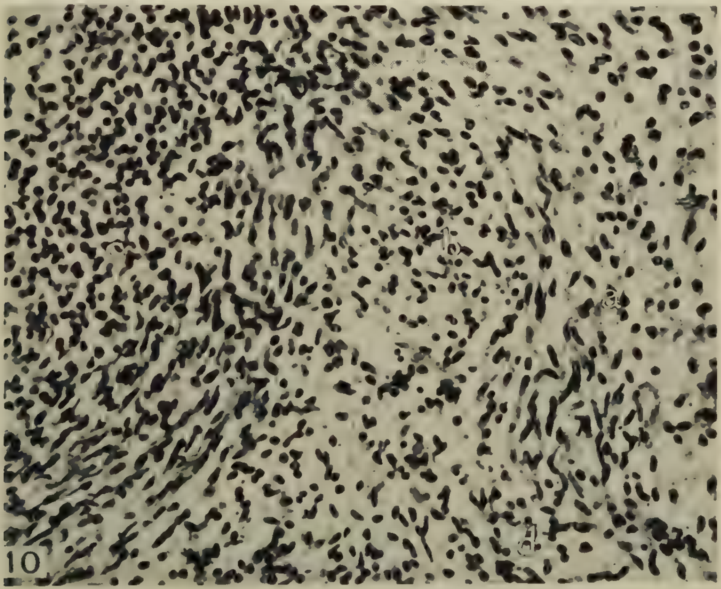


Fig. 9 Photograph of a transverse section of the right shoulder of a 22-mm. (V.B.) embryo: a, the mesenchyme from which the capsule of the joint will be formed; b, a cross section of the long tendon of the biceps; c, the head of the humerus; d, the tip of the coracoid process. × 225.

Fig. 10 Same as figure 9, except × 400. To identify the fibers marked (b) as a cross section of the long tendon of the biceps, serial sections were followed till these fibers were seen to attach to the biceps muscle.

In the transverse section of the shoulder joint of a 28-mm. fetus (fig. 8), it may be seen that the fibers making up the long tendon can be clearly differentiated from the surrounding tissues. The tendon is enclosed within a sheath made up of a few fibers of circularly arranged connective tissue, not closely applied to it, there being clear spaces around the tendon, which lies close to the head of the humerus and is separated from it only by the fibers of the connective tissue sheath. The joint capsule is represented by a group of fibers which are not sharply demarcated from the surrounding mesenchyme but which may be seen in figure 8 as passing around the humerus and over the long tendon and blending with the fibers of the outer side of the sheath of the tendon.

Figures 9 and 10, which are from transverse sections from the shoulder of a 22-mm. embryo, represent a very early stage in the development of the structures concerned. The joint cavity has not formed and mesenchyme surrounds all of the differentiated structures. By following it in serial sections from its attachment to the biceps muscle, the structure labeled (b) in figures 9 and 10 could be identified as the long tendon. It is seen lying close to the humerus and is surrounded on its outer surface by the mesenchyme from which the joint capsule is just beginning to differentiate.

The relationships of the long tendon of the biceps to the capsule of the shoulder joint and other neighboring structures may be summarized as follows. Late in the second month, according to Streeter's ('20) tables, the long tendon can be identified and the beginning of the formation of the joint capsule seen. At this time the tendon lies close to the humerus, and the fibers of the capsule extend over its outer surface, thus enclosing it before the joint cavity is present. During the first week of the third month, the tendon is enclosed within a sheath of connective tissue fibers loosely arranged around it. Only the fibers of the synovial sheath separate the under surface of the tendon from the humerus; and the joint capsule to the under surface of which the sheath of the tendon is attached, extends over the outer surface of it. By the middle of the third month, the joint cavity has appeared and the tendon is surrounded by its sheath which separates it from the joint cavity. The sheath is closely attached to the under surface of the capsule and is composed of cells and fibers indistinguishable from those lining the joint cavity. In the third week of the third month the tendon shows considerable increase in size and its sheath is more closely applied to it. The connection of the sheath of the tendon to the capsule becomes less intimate because of an increase in the length of the attachment uniting them. This separation of the sheath of the tendon from the under surface of the capsule continues, and late in the third month the attachment between them has become much longer and thinner.

In all stages of development studied, the long tendon of the biceps lay under, i.e., within and not without, the capsule of the shoulder joint. I never found it even partly outside of the capsule or in a position which made its relation to the capsule doubtful. As soon as the tendon could be identified, it lay next to the head of the humerus with the fibers of the developing joint capsule over it. A primary extracapsular position of the tendon with subsequent migration of it through the capsule to a secondary position within the joint cavity hence has not been confirmed.

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THE BONE MARROW IN THE MONKEY (MACACUS RHESUS)

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ONE TEXT FIGURE AND ONE PLATE (THREE FIGURES)

Although the hematopoietic function of the bone marrow has been known for considerably more than 50 years, the anatomy of the organ and its physiologic and pathologic significance, are still incompletely understood. Its diffuse distribution and its position in the body have rendered its study more difficult than that of most organs. Consequently, questions of cellular potency and of circulatory pattern remain unsettled.

Recently clinical examination of bone marrow in certain of the myeloid and erythroid dycrasias has given much valuable data on the qualitative distribution of marrow cells in these diseases. Such examinations are of necessity restricted to a single locus, usually the sternal marrow, where material for biopsy is readily obtained. Recent experimental work, however, has shown that different portions of the bone marrow react alike to comparable stimuli, so that conditions found at biopsy of any given region are likely to be characteristic of those elsewhere in the marrow.

Data recently assembled, concerning both man and experimental animals, show that under normal conditions bone marrow from greatly isolated regions of the body is essentially alike in the qualitative and quantitative distribution of its cells. Nordenson studied bone marrow of man, from the sternum, the iliac crest, a lumbar vertebra and the tibia, and found a marked identity in the cellular distribution in these different regions. Yamomoto ('25) examined the marrow of nine different foci in rabbits and arrived at the same conclusion as Nordenson. Likewise Sabin and Doan ('27) found no constant differences in the cytology of different portions of marrow of the rabbit. Williams ('35) studying material obtained routinely at necropsy, examined five portions of marrow of man and he too confirmed the work of Nordenson. We ('35) examined the marrow of the femurs and ribs of rats and found that total myeloid activity of the femur slightly exceeded that in the rib, although marrow of the rib exceeded that of the femur in total erythroid production. Likewise, we ('36) have studied the bone marrow of the ribs and of two regions of the femur in a series of thirty-five dogs and have found comparable conditions in the three regions. Percentages of erythroid cells, however, exceeded percentages of myeloid cells in all three loci. Thus, clinical post-mortem and experimental data point to the conclusion that bone marrow, although diffuse and widely scattered over the body, functions as a unit.

Furthermore, marrow of the various regions reacts alike to given stimuli. When we examined the bone marrow of rats in which we ('36) had induced hepatic cirrhosis accompanied by macrocytic anemia, we found strikingly coordinated changes in the bone marrow of the femurs and ribs. Under the conditions of that experiment there was a suppression of the myeloid line and a stimulation of the erythroid line; but comparable changes occurred in the marrow of both regions examined.

The removal of the spleens from white rats will, in Bartonella-carrier stock, induce a marked anemia which reaches its peak in about a week. We ('36) examined the bone marrow in the ribs and femurs of a large number of splenectomized rats and found an erythroid stimulation in both regions. Erythroid activity in the rib of the normal rat is always greater than in the femur and during the marked stimulation after splenectomy, the rib still exceeded the femur in this respect, except on the fifth day after operation.

Quantitative data on the cytology of bone marrow is almost imperative if one is properly to evaluate degrees of cellular stimulation that a given condition has induced. One may regard a specimen of bone marrow as giving evidence either of hypoplasia or of hyperplasia and yet, unless counts are made and a base line has been established, adequate appraisal of the degree of change cannot be made. Such quantitative data are now available for the normal rabbit, chicken, man, rat and dog. Since the monkey is being used more and more as an experimental animal we have concluded a comparable study on the marrow of this primate which may serve as a basis for future investigations. This report covers a study of the percentage distribution of the cells in the bone marrow of the monkey, Macacus rhesus.

MATERIAL AND METHODS

Six adult monkeys, which had been housed in the laboratories at the Institute of Experimental Medicine for 3 years were used in this study. Immediately after death, bone marrow was taken from five different regions; the sternum, rib, vertebra, right femur and right tibia. Specimens of marrow were fixed in Helly's fluid and paraffin sections were stained with Dominici's stain. In addition, imprint preparations of these five portions of marrow were made on clean slides in a manner hitherto described ('35); the slides were quickly dried by whipping them through the air and then were stained with the May-Grünwald-Giemsa stain. The paraffin sections were made for comparison with the imprint preparations but all counting and computation of the percentage distribution of the various cells were made from the stained imprint preparations. Five hundred cells were counted from the preparations made from each portion of marrow, so that 2500 cells were counted from each animal. A field, representative of the imprint preparations from which our counts were made, is shown in figure 2.

OBSERVATIONS

We have not taken the space required to describe the various cells which comprise the myeloid and erythroid series as they occur in the bone marrow of monkeys. We described these cells as they appeared in our imprint preparations of the marrow of white rats and of dogs, and they have been adequately described for man. Since there is a general similarity in the appearance of these cells it has seemed best to us to omit all descriptions from this brief report.

In general, the morphologic patterns of the bone marrow of man, of the monkey and of the dog are essentially alike. In the dog, however, the neutrophilic granules in the myelocytes and metamyelocytes stain very faintly, but otherwise there are no morphologic criteria whereby the cells of these preparations of marrow can be identified, the one from the other. Hall found in his study of the blood of the monkey that the nuclei of the polymorphonuclear cells were more extensively lobulated than those of the blood of man. We did not recognize any significant difference in this regard in the nuclei of granulocytes as they appeared in our preparations of marrow.

On the basis of its gross appearance, marrow in the adult is often described as 'red' or 'yellow.' Red marrow is considered to be actively hematopoietic and occurs in the skull, sternum, ribs, vertebrae and proximal portions of the long bones. Yellow marrow is so described because of its content of fat and it is the opinion that formation of blood has ceased in those regions where fat has accumulated. Sites of yellow marrow are the extremities, such as the tail and distal portions of the femur and tibia. It is probable that fat abounds in varying degrees in all portions of marrow, depending on either physiologic or pathologic stimuli. In hypoplastic marrow the preponderance of fat will be greater than in the presence of an anemic condition or than in regions of low oxygen tension where hyperplasia of the marrow is likely to occur.

Three of the five specimens of bone marrow examined, those from the sternum, from a rib and from a vertebra, were

TABLE 1

Percentage distribution of the cells in five regions of the bone marrow of the monkey

	MYELOBLAST	LEUKOBLAST	PROMYELOCYTE	MYELOCYTE	METAMYELOCYTE	GRANULOCYTE	EOSINOPHIL	NORMOBLAST	LYMPHOCYTE	RETICULOENDOTHELIAL CELL	MEGAKARYOCYTE
Sternum											
Mean	0.27	1.99	3.02	9.35	17.92	21.22	2.06	40.78	2.47	0.64	0.17
Probable error	±0.09	±0.11	±0.26	±0.71	±0.96	±1.13	±0.42	±0.81	±0.20	±0.32	±0.15
Sigma	0.34	0.41	0.95	2.57	3.50	4.11	1.52	2.95	0.73	1.17	0.55
Coefficient of variation	125.9	20.6	31.5	27.5	19.5	19.4	73.8	7.2	29.6	182.8	323.5
Rib											
Mean	0.35	1.70	2.35	8.65	17.62	21.16	2.13	42.11	2.56	0.89	0.34
Probable error	±0.04	±0.14	±0.22	±0.43	±0.39	±0.53	±0.37	±0.44	±0.21	±0.11	±0.05
Sigma	0.16	0.53	0.82	1.57	1.43	1.92	1.33	1.60	0.76	0.40	0.18
Coefficient of variation	45.7	31.2	34.9	18.2	8.1	9.1	62.4	3.8	29.7	44.9	52.9
Vertebra											
Mean	0.15	1.66	2.95	8.68	17.52	22.68	2.10	40.91	2.40	0.65	0.21
Probable error	±0.09	±0.12	±0.29	±0.62	±0.79	±0.85	±0.31	±0.95	±0.08	±0.09	±0.04
Sigma	0.33	0.45	1.05	2.25	2.87	3.09	1.14	3.43	0.28	0.35	0.17
Coefficient of variation	220.0	27.1	35.6	25.9	16.4	13.6	54.3	8.4	11.7	53.8	80.9
Femur											
Mean	0.33	1.59	2.89	6.90	14.95	21.45	2.20	45.20	2.28	0.93	0.26
Probable error	±0.09	±0.08	±0.28	±0.58	±1.28	±1.13	±0.38	±1.70	±0.09	±0.10	±0.08
Sigma	0.33	0.31	1.02	2.10	4.64	4.10	1.38	6.19	0.33	0.36	0.31
Coefficient of variation	100.0	19.5	35.3	30.4	31.0	19.1	62.7	13.7	14.5	38.7	119.2
Tibia											
Mean	0.19	1.51	2.64	6.28	14.00	19.56	1.66	49.72	2.95	1.12	0.25
Probable error	±0.08	±0.10	±0.23	±0.21	±1.18	±1.18	±0.25	±1.20	±0.14	±0.13	±0.06
Sigma	0.28	0.36	0.85	0.77	4.29	4.28	0.92	4.34	0.51	0.47	0.21
Coefficient of variation	147.3	23.8	32.2	12.3	30.6	21.9	55.4	8.7	17.3	41.9	84.0

definitely red and the gross appearance did not suggest the presence of fat; but the middle of the shaft of the femur and the tibia, especially, were yellow, containing varying amounts of fat.

Survey of table 1 and of figure 1 indicates that in spite of this difference in the gross appearance of the bone marrow of these regions, there are no very great differences in the percentage distribution of the cells. A comparison of the data compiled for the cellular distribution in the marrow of the sternum, a rib and a vertebra shows that there are no significant differences in the percentages for any of the cell groups. Likewise, when the percentages of the cells of the marrow of the femur are compared with those of the marrow of the tibia they too are found to be statistically alike. However, when the percentages computed for the cells of the marrow of a flat bone, for example the sternum, are compared with those for the cells of the marrow of the femur or of the tibia some

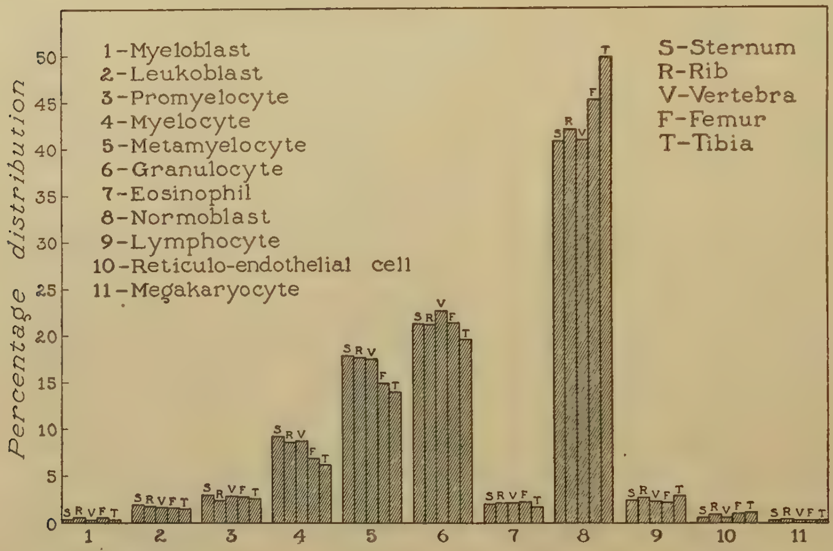

Fig. 1 Percentage distribution of the various cells found in the five regions of the bone marrow of the monkey.

differences that are of statistical significance are noted. These differences pertain particularly to certain of the myeloid forms and to the normoblast series (table 2). In general, the percentages of myeloid cells, especially leukoblasts, myelocytes, and metamyelocytes, are lower in the marrow of the femur and of the tibia than in the sternal, rib or vertebral marrow. The percentages of normoblasts on the other hand, are significantly higher in the long bones, especially the tibia, than in either the sternum, a rib or a vertebra. In other words a preponderance of myeloid activity occurs in the flat bones, whereas erythroid activity predominates in the long bones.

TABLE 2

Significant differences between the percentage distributions of cells in the bone marrow of the sternum and those of the femur and tibia

CELL	FEMUR	TIBIA
Myeloblast	N.S.	N.S.
Leukoblast	S.(D)	S.(D)
Promyelocyte	N.S.	N.S.
Myelocyte	S.(D)	S.(D)
Metamyelocyte	N.S.	S. (borders) (D)
Granulocyte	N.S.	N.S.
Eosinophil	N.S.	N.S.
Normoblast	S. (borders) (I)	S.(I)
Lymphocyte	N.S.	N.S.
R. E.	N.S.	N.S.
Megakaryocyte	N.S.	N.S.

N.S. = no significant difference.
S.(D) = significant decrease.
S.(I) = significant increase.

When the myeloid-erythroid ratios are computed this contrast is again clearly shown (table 3). In the flat bones, the ratio of myeloid percentages to erythroid percentages was considerably more than 1, while in the femur it was but slightly greater than 1 and in the tibia it was less than 1. In rats, rabbits and man the myeloid-erythroid ratios were found to be greater than 1, while in our series of dogs we found the myeloid-erythroid ratio in two regions of the femur as well as in the rib always less than one, indicating a preponderance of erythroid activity.

The preponderance of erythroid activity in the tibial marrow of the monkey is of interest, when it is considered that this marrow is so largely fatty. But interspersed with the portions of fat are the many hematopoietic foci (fig. 3). As Askanazy ('27) stated, there is no sharp line of demarcation between the red and the fatty marrows, but in the tibial marrow of the monkey these hematopoietic foci occur more largely in the peripheral rather than in the central portions of the marrow.

TABLE 3

Myeloid-erythroid ratios in various regions of the bone marrow of the monkey, Macacus rhesus

BONE	PER CENT			
	Total myeloid cells	Total erythroid cells	Lymphocytes	Myeloid-erythroid ratio
Sternum	56.00	40.78	2.47	1.37:1
Rib	54.30	42.11	2.56	1.28:1
Vertebra	55.95	40.91	2.40	1.36:1
Femur	50.57	45.20	2.28	1.11:1
Tibia	46.09	49.72	2.95	0.92:1

As the marrow becomes hyperplastic these small hematopoietic loci proliferate, increase in size and the areas of fat correspondingly decrease as the red marrow increases in extent. This is well illustrated in the case of one of the monkeys in which the total erythrocyte count was slightly lower than in the other five. Here there was some hyperplasia of these hematopoietic islands (fig. 4) and the amount of fat was correspondingly reduced.

COMMENT

This study of the quantitative distribution of the cells in the bone marrow shows that in the monkey as well as in man and other mammals, there is marked similarity in the percentages of the cells of the various regions of marrow examined. Although the monkeys had been confined in the laboratory for

3 years, yet their mean erythrocyte count was 5,125,000 per cubic millimeter and we had every reason to regard them as completely normal.

The study sustains Bock ('32) who investigated the cellular output from different portions of bone marrow, using the heart-lung preparation. He concluded that although changes were somewhat parallel, yet granulocytopoiesis exceeded erythropoiesis in the flat bones; the reverse was true in the long bones. In the monkey we found that myelopoiesis exceeded erythropoiesis at all levels examined except the tibia, a region predominantly fatty, where the percentage of erythroid cells was only slightly in excess of that of the myeloid group of cells.

It is generally true, under normal physiologic conditions, that the relative number of myeloid cells in bone marrow exceeds that of erythroid cells. Myeloid cellular activity is greater than production of erythroid cells. Myeloid-erythroid ratios, therefore, are normally greater than 1. In the literature, reports of this ratio vary considerably and no doubt hinge on the exact physiologic condition of the animal or person whose marrow was studied. Animals accepted as normal or healthy, may well harbor conditions which may serve as centers of stimulation, either in the myeloid or erythroid directions and thus the myeloid-erythroid ratio may be modified. It is difficult at times to determine where physiologic fluctuation ends and pathologic proliferation begins. Young and Osgood ('35) found a ratio of 3.61:1 in man, while Schilling ('25) reported a ratio of 1.21:1. Sabin and Doan reported a ratio of 2.6:1 for rabbits while we found a ratio of 1.75:1 in the femur and 1.40:1 in the ribs of rats. In the dog our ratios for the rib and for two regions of femur marrow were all less than 1, due of course to a preponderance of erythroid activity, thus inverting the myeloid-erythroid ratio. We cannot account for this difference in the dog. Our dogs comprised a series of animals used for experimental purposes and since erythrocyte counts were not made we cannot state that a slight anemia may have induced this erythroid preponderance.

Our data concerning the monkey fit in fairly well with Schilling's data concerning man. The myeloid-erythroid ratio was highest in the sternum and lowest in the tibia. It was greater than 1 at all levels examined except the tibia.

SUMMARY AND CONCLUSIONS

This report covers a study of the bone marrow taken from five different regions of the monkey, Macacus rhesus. Both imprint preparations and paraffin sections were made and differential counts were made of the various cells in the marrow.

The following points seem clearly established:

1. There is a marked similarity in the percentage distribution of the various cells throughout the bone marrow.
2. There is a preponderance of myeloid activity in the sternum, rib, vertebra and femur, but the tibia excels in production of erythroid cells.
3. Although the marrow in the shaft of the tibia is predominantly yellow, because of its fat content, yet numerous hematopoietic foci are scattered throughout the fatty areas. These are largely restricted to the subosteal regions of the marrow. They may increase in size and result in a hyperplastic condition of the marrow, reducing thereby the amount of fat.
4. The myeloid-erythroid ratios are greater than 1 in all sites examined except the tibia.

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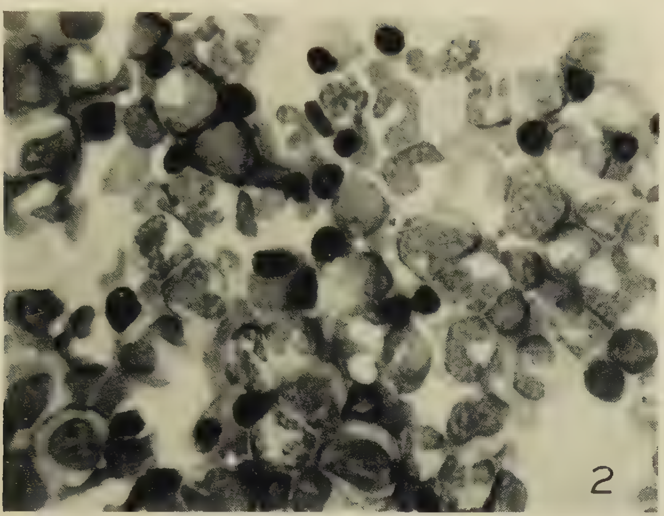
PLATE 1

EXPLANATION OF FIGURES

2 Imprint preparation of the marrow of sternum of monkey, Macacus rhesus. May-Grünwald-Giemsa stain. × 1000. Representative cells of myeloid and erythroid series are shown.

3 Section of bone marrow of tibia of monkey, Macacus rhesus. Helly fixative; Dominici stain. × 60. Hematopoietic foci distributed throughout fat areas.

4 Section of bone marrow of tibia in which there was some stimulation of hematopoietic foci. Helly fixative. Dominici stain. × 435.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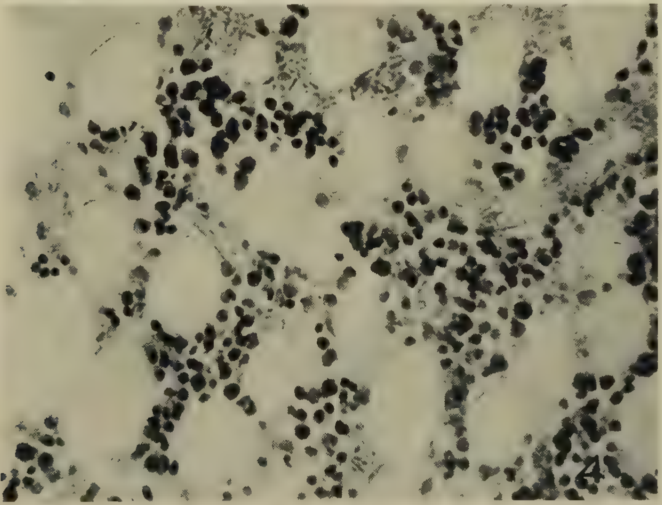
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REPRODUCTIVE ORGANS OF TWO MAMMALIAN HERMAPHRODITES AND THEIR RESPONSE TO INJECTIONS OF PREGNANT MARE SERUM[1]

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TWO PLATES (THIRTY FIGURES)

INTRODUCTION

The sporadic occurrence of intersexuality in mammals accounts for the lack of systematic study of mammalian hermaphroditism. This is especially true in man. Most cases of human hermaphroditism are discovered either in youthful individuals, in which a complete analysis of their reproductive condition is impossible, or in autopsy individuals whose genital organs are too poorly preserved for adequate detailed study.

The intersexual character of two ground squirrels (Citellus tridecemlineatus) was detected at exploratory laparotomy. Observations on these two animals have been abstracted elsewhere (Wells, '36 b). Since reproductive organs in each animal were in a low state of activity, one animal was given injections of gonadotropic material in an attempt to stimulate whatever gonad tissue was present; the other individual was autopsied as untreated control. Gross and microscopical studies of reproductive organs from these two animals form the basis for this paper.

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[2] Much of this work was done in the Hull Zoological Laboratory at The University of Chicago.

The writer is greatly indebted to Dr. Carl R. Moore for helpful advice, and to Dr. G. H. Hart of Davis, California, who supplied the blood serum from pregnant mares.

MATERIALS AND METHODS

Normal ground squirrels are in a breeding condition only during the annual rutting period occurring in April and May. At other seasons of the year the entire reproductive tract is in a sub-breeding state which is characterized by aspermatic testes and non-functional accessories in the case of the male (Wells, '35 a) and by anoestrus in the case of the female (Moore, Simmons, Wells, Zalesky and Nelson, '34).

A brief statement concerning the discovery of the two hermaphrodites is probably in order, since both animals gave an external appearance and body weight characteristic of normal males and might possibly have evaded detection until autopsy. Until a recent date the writer was unable to recognize external criteria for determining, during late summer and autumn, whether any particular animal of this species was either immature (born approximately June 1st) or non-rutting adult. Internal reproductive organs gave definite evidence as to the age of the animal, therefore, all males collected from the field were routinely examined through a peritoneal incision soon after capture and the age in each case was determined. Both hermaphrodites were first detected during the course of such laparotomies.

Observations indicate that hermaphroditism rarely occurs in this species. More than 2000 sub-adult or adult individuals of this species have been collected alive from the field during the interval from 1931 to 1936. The reproductive tract of practically all of the males has been examined at autopsy by the writer, while that of nearly all of the females has been observed by interested collaborators. These two animals are the only hermaphroditic individuals found in the entire group.

One hermaphrodite was collected on October 7, 1934, while the other was collected 1 week later on October 14th from the same field (it is even possible that the two individuals were littermates).

Reproductive organs of both hermaphrodites appeared infantile at laparotomy. If the animals were actually immature, and all indications suggest this to be the case, they were approximately 4 months of age at the time of collection and laparotomy. The infantile condition of the genital organs was not surprising, since normal young of this species do not reach a breeding condition until the spring following that of their birth.

The peritoneal incision of one animal, upon discovery of a hermaphroditic condition in the genital tract, was immediately closed without removal of any reproductive tissue. This animal was allowed 3 days to recover from the operation and was then given injections of pregnant mare serum. Subcutaneous injections in doses of 25 rat units were made twice daily for 18 days until autopsy which occurred on November 3, 1934. The other hermaphroditic animal was killed without treatment immediately after laparotomy on October 7, 1934.

The reproductive tract of each animal was carefully dissected in toto, caution being taken to leave much superficial fat so as to prevent the accidental destruction of possible rudimentary organs. Measurements of the larger reproductive organs were made by means of a sliding caliper. None of the organs were weighed, since such procedure might have resulted in mutilation of questionable structures. The entire tracts were fixed in toto in Bouin's solution and photographed. The entire genital system of each animal was sectioned serially at 10 μ, following dehydration with dioxan and embedding in paraffin. The sections were stained with Ehrlich's hematoxylin and eosin, after which they were utilized in microscopical studies.

ANATOMICAL OBSERVATIONS AND THE RESULTS OF INJECTIONS

In each animal, the male accessory reproductive organs (with the exception of absence of the prostate) showed a remarkable similarity to those in normal males (figs. 1, 3, 5). Female accessories were fairly typical of those found in normal females, but, as will be shown below, the vagina was atypical of that in normal females. It is thought, therefore, that both animals were possibly genetical males in which a gonadal cortex formed and the mullerian system persisted.

Degree of gross development of the reproductive system proved by objective inspection at laparotomy to be equal in each of the animals. It should be stressed, therefore, that the untreated animal served as an ideal control to the injected individual, and that the marked difference of the two reproductive tracts at autopsy was unquestionably due to the treatment of one animal with male serum.

Ovotestes at laparotomy were extremely small and were located bilaterally between the inguinal canal and the posterior extent of the peritoneal cavity of each animal.

In the untreated animal, little difference could be detected between right and left gonads. Though bilateral scrotal pouches were present, the gonad complex lay in an anterior inguinal position (fig. 1). The medullary part of each gonad consisted of seminiferous tubules and interstitial tissue, and occupied approximately 80% of the total volume of the ovotestis (figs. 16, 22). Seminiferous tubules were similar to those found in normal immature males of this species, but they were separated by a much greater amount of interstitial tissue and fibrous connective tissue than one encounters in normal males. The seminiferous tubules lacked lumina, but possessed many spermatogonia and occasional primary spermatocytes (figs. 17, 23). Junction of seminiferous tubules, tubuli recti and rete tubes was observed in both gonads. Ovarian cortex occupied the antero-lateral part of each ovotestis, forming a sort of cap for the medulla. Presence of primordial ova on the surface of the gonads was evidence that the germinal epithelium was active. Growing follicles with the granulosa layer varying from one to four cells in thickness were embedded in the ovarian stroma; cytoplasm and germinal vesicle of the ova appeared morphologically normal (figs. 19, 26). Neither follicles with cavities nor corpora lutea were present.

The injected animal, whose gonads macroscopically resembled those of the untreated control at laparotomy, showed greatly enlarged ovotestes at autopsy (fig. 3). Injections caused the right ovotestis to enlarge and descend to its scrotum, but failed to cause descent of the left ovotestis. The left ovotestis, enlarged in response to treatment, attained a size approximately half as great as the right. The *right gonad* contained testicular elements to the extent of about 95% of the entire volume, the remainder of the volume being composed of ovarian elements (fig. 15). Morphology of the testicular portion strongly resembled that of the testis of a normal breeding male. Seminiferous tubules contained all elements of the germinal epithelium, including spermatozoa which were unquestionably formed in response to gonadotropic materials present in the injected mare serum (fig. 18). Rete tubes were present but they failed to communicate with the epididymis. This gonad exhibited few primary follicles, many follicles with small cavities, apparently mature follicles and nine large corpora lutea (fig. 20). Retained ova were seen in the graafian follicles and in the corpora lutea. Ovarian stroma showed marked atypical luteinization. The *left ovotestis* contained a greater amount of ovarian elements than testicular components (fig. 21). It exhibited many primary follicles, follicles with both small and large cavities and corpora lutea, retained ova being found in the graafian follicles and corpora lutea (fig. 25). The atypical luteinization of ovarian stroma, mentioned above, was also noted in this gonad. Seminiferous tubules were few in number, but they appeared morphologically normal and essentially infantile. The tubules contained many spermatogonia and atypical clumps of primary spermatocytes, but they lacked the elements of advanced spermatogenesis (fig. 24). Failure of this gonad to form spermatids and spermatozoa in response to injections, was probably due to the fact that descent to the scrotum did not occur on this side of the body. Rete tubes were present, but they failed to unite with the epididymis.

Epididymides (figs. 15, 16, 21, 22) in both animals were morphologically divisible into head, body and tail regions. In the uninjected animal there was a patent passage from rete tubes to vasa efferentia and from vasa efferentia to caput epididymis. In the injected animal, however, rete tubes failed to join with the epididymis both on the right and on the left sides of the body. The histological structure of the epididymis appeared normal in each case, yet the epididymides of the untreated animal were essentially infantile and those of the injected individual strongly resembled epididymides of breeding adults. Spermatozoa were not found within the lumen of the epididymides; spermatozoa were not produced by three of the ovotestes, and those spermatozoa formed within the right ovotestis of the injected animal were prevented from reaching the corresponding epididymis by morphological blockage.

Ductuli deferentes had patent communication with epididymides in each animal, and they showed no occlusion at any point. Ductuli of the injected animal showed both gross and histological evidence of stimulation (figs. 29, 30), so that they appeared histologically similar to those of breeding males. Ducts of the control hermaphrodite were histologically normal, but they were in an infantile state.

Each ductus paralleling a uterine horn, ran toward the median plane of the body to a point near the urinary bladder. The two ductuli, now in positions lateral to the corpus uterus, enlarged into ampullae. Each ductus deferens fused with a seminal-vesicle duct to form paired ejaculatory ducts, which failed to pierce prostatic tissue. Ejaculatory ducts of the injected animal fused in the median plane of the body, and joined at the point of fusion with the cavities of both vagina and urethra (fig. 8). Ejaculatory ducts of the untreated animal emptied into the vagina (figs. 10, 11, 12).

Urethra of each hermaphrodite resembled in its pattern that of a normal male more than that of a female. Three general regions could be distinguished: 1) A proximal pseudoprostatic portion lined by 'transitional' epithelium, 2) a pars membranacea surrounded by a thick layer of skeletal muscle, and 3) a pars cavernosa located in the penis and receiving the 'penile duct.' Vagina fused with the pars membranacea in each animal, this fusion being characterized by the fact that both vagina and urethra previous to their junction became enclosed within a common coat of skeletal muscle (figs. 8, 12).

Seminal vesicles of normal histological appearance were present in each hermaphroditic animal. They were small in the uninjected individual and very large in the treated animal. Their gross and histological condition in the injected hermaphrodite was short of the maximum development seen in breeding adults; however, functional activity, characterized by active epithelium and the storage of quantities of secretion in the lumina, had been induced by the mare serum (figs. 27, 28). Seminal vesicle ducts fused with the appropriate ductuli deferentes at the ampullae in each animal.

Prostate gland was absent in each hermaphrodite. Precautions at autopsy dissection of the reproductive tracts were such that there was no possibility of overlooking the prostate gland, had there been one present. Furthermore, no prostatic tissue was detectable in serial sections of the entire genital tract of each animal. Significance of the absence of the prostate gland in these two hermaphrodites will be considered in the discussion of this paper.

Cowper's glands with normal morphology lay in the position usual for males of this species, opposite the pars membranacea urethra. They were in an infantile condition in the untreated hermaphrodite. Cowper's glands in the injected animal, although not as large as those found in rutting males, had attained a high state of functional activity, degree of activity being determined by gross size, height and granular character of the epithelium and storage of large amounts of secretion (figs. 13, 14). Paired Cowper's ducts coursed mediad, but they ran parallel to each other for a great distance before they fused to form the penile duct. Penile duct emptied into the cavernous urethra within the distal third of the penis. See also Mossman, Lawlah and Bradley, '32; Siddiqi, '36.

Bulbar gland (figs. 1, 3) with a normal histological structure was present as an expansion of Cowper's ducts. As true for other male accessories, the bulbar gland of the untreated animal was infantile, while that of the injected hermaphrodite was large and in a functional state somewhat short of the maximum rutting condition for this species.

Penis was histologically normal, and at laparotomy it was in each hermaphrodite approximately the size of that for normal immature males of this species (fig. 1). All structural details of the penis, including the presence of many spines on the glans, agreed with those for the penis of normal animals. Size of the penis of the injected animal was increased by mare-serum treatments, but it was at autopsy smaller than that of breeding males (figs. 3, 5).

Fallopian tubes in each hermaphrodite were present on both sides of the body (figs 15, 16, 21, 22). Each tube was provided with an ostium tuba which communicated with the peritoneal cavity. The left fallopian tube of each animal had a patent lumen throughout its course and joined with its corresponding uterine horn. The right tube of each hermaphrodite had a patent lumen both at the ostium end and at the uterine end, but the middle portion of the tube was entirely absent. Coils of the fallopian tubes were in contact with the epididymis.

Cornua uteri of each animal ran at almost right angles toward the median plane of the body, coursing parallel to their corresponding ductuli deferentes (figs. 29, 30). Uterine glands, epithelium and muscular coats appeared microscopically normal. Uterus of the untreated hermaphrodite showed no signs of hormonal stimulation. Gross and histological criteria indicated that the uterus of the injected animal had been subjected to the action of oestrogenic material, since the endometrium alone was as thick as the entire uterine diameter for the uninjected hermaphrodite and the myometrium was practically as thick as the endometrium. No evidence of a 'corporin effect' on the uterus was detectable, although many corpora with normal histological morphology were present in the ovotestes of the hermaphrodite receiving mare-serum injections.

Corpus uterus was present in each hermaphrodite; but, as in normal females of this species, lumina of the two uterine horns did not fuse to form a single uterine cavity. The lumen of each uterine horn opened through separate cervices into the vagina.

Vagina, lacking an external genital orifice and situated within the skeletal-muscle coat of the urethra, was present in each hermaphrodite. The vagina of the untreated animal opened broadly and directly into the membranous urethra (fig. 12), while that of the injected animal communicated indirectly with the membranous urethra at the point of fusion and entrance of the ejaculatory ducts; i.e., cavities of the vagina, ejaculatory ducts and membranous urethra communicated at a common point (fig. 8). Distal to this point, the vagina of the latter animal ended blindly (fig. 9). Vagina of the uninjected individual showed no signs of hormonal action, while that of the treated hermaphrodite exhibited marked epithelial cornification characteristic of oestrus.

Other organs of these two hermaphrodites were studied by interested collaborators who have had wide experience with these organs in normal ground squirrels and have authorized the writer to record their observations. These studies were: Dr. Warren O. Nelson of Yale University, the pituitaries; Dr. Moses Zalesky of The University of Chicago, the adrenals and thyroids; and Mr. E. T. Gomez of the University of Missouri, the mammary glands. The writer quotes below the results of these studies.

> Neither the thyroids nor the adrenals of these two hermaphrodites showed any pathological growth or other structural peculiarities. Thyroids and adrenals of the untreated animal were typical of those in normal animals of this species during the period of sexual quiescence. The thyroids of the injected animal differed in no wise from those of the control hermaphrodite. Adrenals of the injected animal weighed 250% more than those of the control hermaphrodite. There was a pronounced hypertrophy of the adrenal cortex in the injected animal, the outer reticularis characteristic of breeding ground squirrels being highly developed.—Zalesky.

Pituitaries of these animals exhibited no evidence of pathology. Hypophyses of both animals showed a higher percentage of small basophils than that usually encountered in this species; however, the writer is not inclined to relate this phenomenon to the hermaphroditic condition of the animals in question. The pituitary of the untreated hermaphrodite histologically resembled that of normal immature animals. The pituitary gland of the hermaphrodite receiving mare-serum resembled that of both breeding ground squirrels and normal immature animals receiving mare-serum injections. —Nelson.

The mammary glands of these hermaphroditic animals showed no evidence of abnormality. The glands of the untreated animal consisted mainly of rudimentary ducts which extend slightly beyond the base of the nipples. Histologically, these structures resemble those found in the immature females or adult males of this species. In the injected hermaphrodite the mammary glands showed growth and arborization of the duct system similar to that obtained during the early stage of estrogenic-hormone stimulation in normal animals. This change was taken to indicate a mild stimulation by the estrogenic hormone elaborated by the ovotestes (?) following their stimulation by the pregnant mare's serum.—Gomez.

DISCUSSION

Studies of mammalian hermaphroditism have recently been reviewed by Witschi ('32) who cites references to about thirty cases of so-called hermaphroditismus verus in man, and by Crew ('26, '27). Some postulations that have been employed in attempting to account for the occurrence of hermaphroditism in mammals are: 1) atavism (Sauerbeck, '09; Jordan, '22), 2) quantitative variation in the strength of 'M' and 'F' genes (Goldschmidt, '31), 3) teratological growth resulting in the suppression of ovarian cortex (Pick, '14), and 4) presence of male hormone in the circulating blood of a genetic female (Lillie, '17).

Each of the two hermaphroditic ground squirrels here described possessed all reproductive organs found in normal females and also all organs characteristic of normal males, with the exception of the absence of external female genitalia and prostate gland. Presence of both testicular elements and perfectly formed male external genitalia is rather convincing evidence that these two animals were genetically-determined males, in which an ovarian cortex developed and the mullerian system persisted. Each of the four above-mentioned postulates falls short of an adequate explanation for the occurrence of these two hermaphroditic ground squirrels, since a) postulates 3 and 4 would necessitate that each hermaphrodite be a genetically-determined female (absence of detectable pathology in suprarenal glands of both hermaphrodites is suggestively relevant in considering the inadequacy of postulate 3), b) atavism could not explain why hermaphroditic differentiation occurred, and c) supportive evidence derived from mammalian studies for Goldschmidt's quantitative theory, which was formulated from a study of insects, has not been reported. Recent experiments by Willier, Gallagher and Koch ('35) on chick embryos, however, may shed some light on this question. These investigators report that, following the injection of oestrogenic materials, the left testis of genetic males was transformed into an ovotestis. Injections also caused the persistence and hypertrophy of oviducts in these males, but failed to modify the wolffian ducts. Wolff and Ginglinger ('35) likewise succeeded in transforming genetic-male chick embryos into intersexual individuals by folliculine injections.

Taking the work of Willier, Gallagher and Koch and that of Wolff and Ginglinger as a clue, the writer wishes to mention a possibility that may have suggestive merit. It is conceivable that these two hermaphrodites, beginning development as genetic males, developed an ovarian cortex peripheral to the testes, and that female hormones released by the ovarian portion of the ovotestes caused the persistence of the mullerian system and inflicted no damage on the wolffian ducts. In this concept, of course, the testes must have exhibited bipotentiality in the formation of a secondary germinal epithelium, the incipient cortex.

These two hermaphrodites are of special interest in view of the observations of Lillie ('17) in the free-martin. In both of the hermaphroditic ground squirrels, presence of bilateral testicular components failed to inhibit the development of ovarian cortex. Also, when the appropriate gonadotropic material (mare serum) was injected, the simultaneous formation of spermatozoa, graafian follicles and corpora lutea occurred in the treated hermaphrodite.

These hermaphrodites also deserve special consideration, since they exhibit the most complete bilateral hermaphroditism thus far recorded for mammals, and since they present evidence favoring the idea that true hermaphroditism can differentiate in mammals beginning their development as genetical males (for a review of data suggesting that true mammalian hermaphrodites begin development as genetical females, Witschi, '32). An intersexual shrew, unquestionably beginning its development as a genetic male, has been reported (Brambell and Hall, '36), yet no ovarian tissue was detected.

Absence of prostate gland in these hermaphrodites is of interest because of its bearing on some current questions concerning the etiology of senile prostatic hypertrophy and the origin of the prostate gland. On the basis of the similarity of experimental response to oestrogenic materials by prostate gland and vagina, the possibility that the prostate gland of normal male rodents may originate from the distal end of mullerian ducts has been considered (Lacassagne, '33; Burrrows and Kennaway, '34; Weller, Overholser and Nelson, '36; Wells, '36 a). The primate utriculus prostaticus, which has a mullerian origin, likewise responds to oestrogenic substances in a similar fashion (van Wagenen, '35; Parkes and Zuckerman, '35; Courrier and Gros, '35). Simultaneous presence of mullerian system and absence of prostate gland in both hermaphrodites, is morphological evidence suggesting that the prostate gland of normal male rodents may be derived from mullerian ducts. Anatomical evidence, that may be considered as antagonistic to this view, has been published by

Korenchevsky and Dennison ('36) who reported the presence of prostatic tissue in female rats (female prostate).

Response of reproductive organs to injections of pregnant mare serum deserves mention. Mare serum caused the precocious production of spermatozoa in the right ovotestis which descended to the scrotum, and it also induced the formation of graafian follicles and corpora lutea in both ovotestes. Production of spermatozoa in the ground squirrel in response to pregnant mare serum treatments (Wells, '35 b; Wells, and Moore, '36) is interesting, since mare serum failed to induce spermatozoa formation in young rats (Moore, '36). Mare serum has been reported (Cole, Guilbert and Goss, '32), however, to increase the number of spermatocytes in young rats. All male accessory reproductive organs of the injected hermaphrodite showed great stimulation, probably due to the stimulation (by gonadotropic substance) of the animal's own ovotestes to release male hormone. Fallopian tubes, uterus, vagina and mammary glands of the treated hermaphrodite exhibited unquestionable signs of stimulation by oestrogenic material which was probably produced by the intact ovotestes. Some oestrogenic substances, however, may have been present in the mare-serum.

SUMMARY AND CONCLUSIONS

1. Each hermaphroditic ground squirrel exhibited *bilateral* ovotestes, scrota, tubuli recti, rete testes, ductuli efferentes, epididymides, ductuli deferentes, vesiculae seminales, ejaculatory ducts, glandulae bulbo-urethrales, ostia tubae and cornua uteri, and *unpaired* urethra (male), glanduli bulbi, penis, corpus uterus and vagina.

2. These animals are thought to be genetic males in which an ovarian cortex developed and the mullerian system persisted.

3. Absence of the prostate gland in both hermaphrodites is morphological evidence suggesting that the prostate gland of normal males may originate from mullerian ducts.

4. Mare-serum injections induced the formation of spermatozoa, graafian follicles and corpora lutea. All male and female accessory reproductive organs of the treated hermaphrodite were stimulated.

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KEY TO ABBREVIATIONS IN FIGURES 1 TO 30

B, bulbar gland
b, urinary bladder
C, Cowper's gland
E, epididymis
e, ejaculatory duct
F, fallopian tube
f, fat body
O, ovotestis
P, prostate gland
p, penis
pu, penile urethra
S, seminal vesicle
s, scrotal muscle
T, testis
U, uterus
u, urethra
V, vagina

PLATE 1

EXPLANATION OF FIGURES

1 Gross dissection of reproductive tract from untreated hermaphrodite (one-half natural size).

2 Same as figure 1, showing both plane and level for sections of the reproductive organs.

3 Gross dissection of reproductive tract from injected hermaphrodite (one-half natural size).

4 Same as figure 3, showing both plane and level for sections of the reproductive organs.

5 Gross dissection of reproductive tract from a male ground squirrel killed during the breeding season. (One-half natural size.)

6 Injected hermaphrodite. Section showing fusion of uterus and vagina at the cervix on the right side of the body. Level V, figure 4. × 6.

7 Injected hermaphrodite. Section showing fusion of both uterine cavities with vagina through paired cervices. Level V, figure 4. × 6.

8 Injected hermaphrodite. Section showing communication of vagina, fused ejaculatory ducts and urethra. Level VI, figure 4. × 16.

9 Injected hermaphrodite. Section near the end of vagina which ends blindly $30\ \mu$ distal to this section. Level VII, figure 4. × 16.

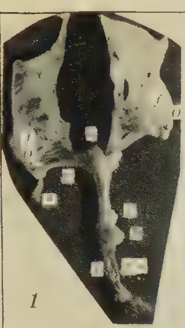
10 Untreated hermaphrodite. Section through vagina, urethra and paired ejaculatory ducts. Level V, figure 2. × 16.5.

11 Untreated hermaphrodite. Section 1.87 mm. distal to figure 10. One ejaculatory duct lies lateral to the vagina and the other communicates with the vaginal cavity. Level VI, figure 2. × 16.5.

12 Untreated hermaphrodite. Sections 4.09 mm. distal to figure 10. Fusion of vaginal and urethral cavities. Vagina and urethra within a common coat of skeletal muscle, as in figure 11. Level VII, figure 2. × 16.5.

13 Injected hermaphrodite. Paired Cowper's glands and urethra. Level VIII, figure 4. × 4.5.

14 Untreated hermaphrodite. Paired Cowper's gland, Cowper's ducts and urethra. Level VIII, figure 2. × 4.5.



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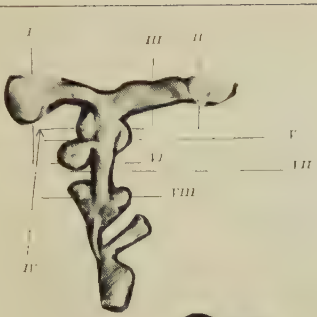
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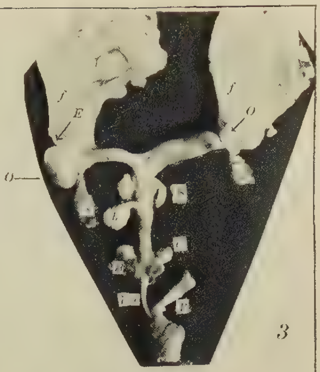

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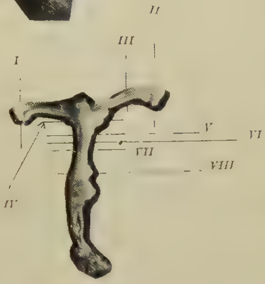
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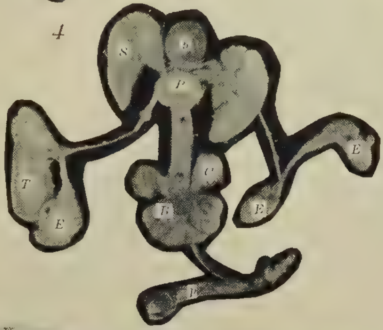

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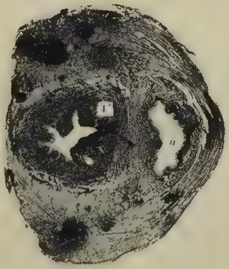

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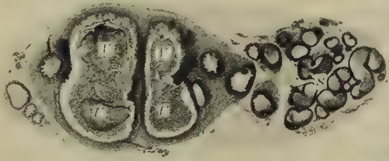

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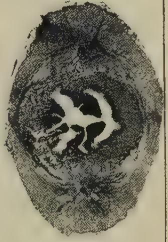

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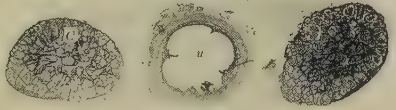

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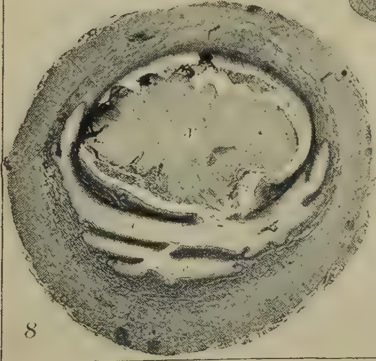

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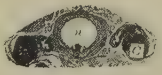

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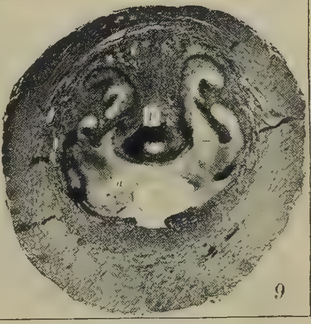

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PLATE 2

EXPLANATION OF FIGURES

15 Injected hermaphrodite. Section of right ovotestis showing largely seminiferous tubules. One graafian follicle and two corpora lutea are shown in lower part of the figure. A small portion of epididymis and fallopian tube may be seen. Level I, figure 4. × 3.5.

16 Untreated hermaphrodite. Section through right ovotestis, fallopian tube and epididymis. Level I, figure 2. × 3.5.

17 Untreated hermaphrodite. Portion of figure 16 showing a higher magnification of seminiferous tubules. Many spermatogonia and few primary spermatocytes are present. × 76.

18 Injected hermaphrodite. Part of figure 15 showing seminiferous tubules at higher magnification. All elements of the germinal epithelium including spermatozoa are present. × 76.

19 Untreated hermaphrodite. Portion of figure 16 showing primary follicles (left in figure) and seminiferous tubules (right). Three primordial follicles located within the two black circles. × 28.5.

20 Injected hermaphrodite. Part of figure 15 showing growing follicles, one graafian follicle and two corpora lutea. × 28.5.

21 Injected hermaphrodite. Section through left ovotestis, fallopian tube and epididymis. Seminiferous tubules, graafian follicles and corpora lutea may be seen. Level II, figure 4. × 8.6.

22 Untreated hermaphrodite. Section through left ovotestis, fallopian tube and epididymis. This section shows only testicular components of the ovotestis (compare with fig. 26). Level II, figure 2. × 8.6.

23 Untreated hermaphrodite. Part of section 22 showing seminiferous tubules. Many spermatogonia and few primary spermatocytes were present. × 125.

24 Injected hermaphrodite. Portion of figure 21 showing seminiferous tubules. Spermatogonia abundant. Spermatocytes atypically clumped in center of most tubules. Spermatids and spermatozoa absent. × 125.

25 Injected hermaphrodite. Part of figure 21 showing seminiferous tubules. One graafian follicle and two corpora lutea may be seen. Growing follicles not shown in this section were present. × 29.5.

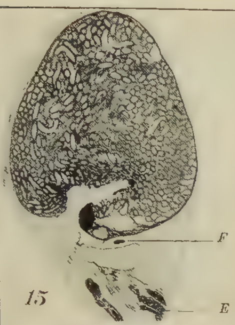
26 Untreated hermaphrodite. Part of left ovotestis showing primordial follicles (indicated by arrows), primary follicles and ovarian stroma. Level II, figure 2. × 29.5.

27 Injected hermaphrodite. Section through paired uteri, seminal vesicles and ejaculatory ducts. Level IV, figure 4. × 6.

28 Untreated hermaphrodite. Section through paired uteri, seminal vesicles and ejaculatory ducts. Level IV, figure 2. × 6.

29 Injected hermaphrodite. Section through the left uterine horn and the left ductus deferens. Level III, figure 4. × 15.5.

30 Untreated hermaphrodite. Section through the left uterine horn and the left ductus deferens. Level III, figure 2. × 15.5.



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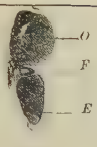

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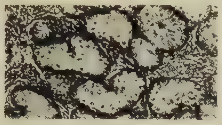

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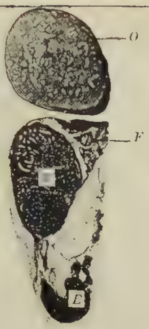

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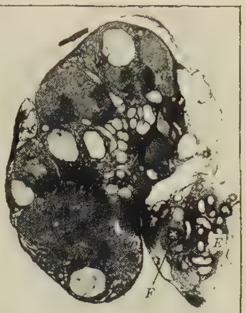

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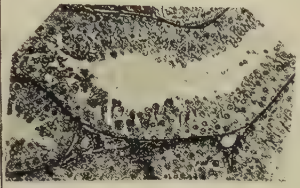

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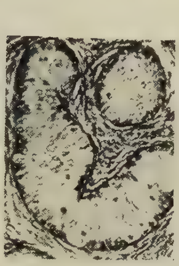

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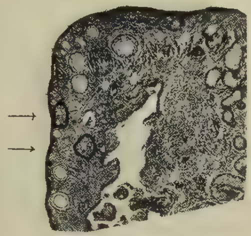

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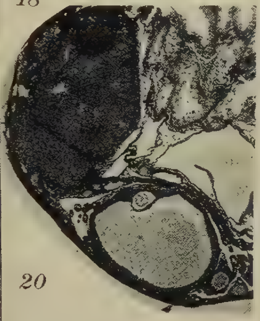

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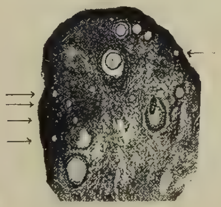

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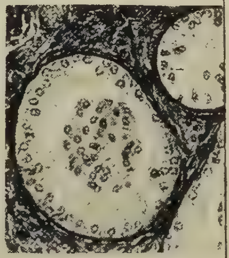

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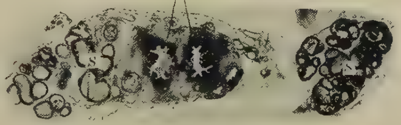

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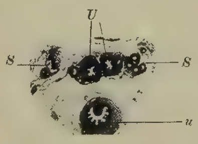

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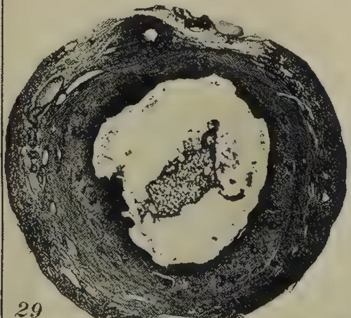

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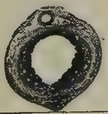

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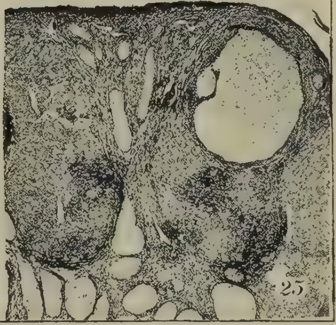

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THE USE OF TRYPAN BLUE IN DETECTING CELL DEATH IN THE PERFUSION OF THE MAMMALIAN KIDNEY, AND THE EVALUATION OF SOME MODIFIED RINGER-LOCKE FLUIDS BY THIS METHOD[1]

JAMES McCOWN DARLINGTON
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THREE PLATES (TWENTY-EIGHT FIGURES)

INTRODUCTION

The interpretation of results of perfusion experiments has been difficult because there has been no adequate criterion of the condition of the cells at the end of the experiment. In general, investigators have employed such criteria as the presence of edema, reaction to stimulation or continuation of function of the whole organ.

Mitochondrial alteration has been used as a criterion of pathological change in cells (Ophüls, '07; Barratt, '13; Lewis and Lewis, '15; Scott, '16; Oliver, '16). The difficulty with this method is that it is not possible to distinguish with certainty between physiological and pathological changes. It was with the hope of determining more accurately the condition of the cells at the end of the perfusion that the vital dye, trypan blue, was used.

The use of this dye in perfusion has not been previously reported. The dye is customarily injected into the animal and the distribution of the dye in the tissues is studied. The dye has red and blue components. The red component, diffusing rapidly, stains diffusely at first but is soon eliminated.

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[1] This paper is a portion of a thesis presented in partial fulfillment of the requirements for the degree of doctor of philosophy at Brown University.

The blue component, if it penetrates cells, is segregated in the form of granules. If a cell is dead or severely injured its cytoplasm and nucleus will be stained a diffuse blue throughout. These facts have been demonstrated by von Möllendorff ('20), McClellan and Goodpasture ('23), Cappell ('29).

Four fluids were used in the experiments here reported. They were Ringer-Locke, Ringer-Locke plus gelatin, Ringer-Locke plus beef red blood cells, and Ringer-Locke plus gelatin plus beef red blood cells. These form a series of fluids graded, theoretically at least, with respect to their physiological adequacy. This was supported by preliminary experiments in which the effect of perfusion on mitochondria and other cell structures was studied. Hence the use of these fluids in a series of experiments designed to test the employment of the dye as a criterion of the relative value of perfusion methods seemed justified.

The writer wishes to express his appreciation to Dr. Walter Wilson for his instructive guidance throughout the course of the study and to Dr. Paul Sawin for furnishing the animals used in the investigation.

MATERIAL AND METHODS

The kidneys of rabbits were used in these experiments.

Experimental apparatus. The perfusion apparatus was designed to control the pressure (mean and pulsatile), temperature, and oxygenation of the perfusion fluid. The fluids were perfused under systolic and diastolic pressures of 110 mm. of mercury and 95 mm. of mercury, respectively, with the consequent pulsatile pressure of 15 mm. of mercury. Sixty-five pulsations per minute were imparted to the fluid. The temperature of the fluids was maintained within a $\frac{1}{2}$°C. of 37.5. The kidney, during perfusion, was kept in a constant temperature chamber regulated to 37.5°C. Complete oxygenation was assured by the constant bubbling of medical oxygen through the perfusion fluid during the process of perfusion. The hydrogen ion concentration of the fluids was determined by a glass electrode. The pH of the fluids ranged from 7.3 to 7.5 with the majority within 0.03 of a unit of pH 7.4.

In the preparation of the fluids chemically pure salts and glass distilled water were employed. The gelatin used was an isoelectric product of the Eastman Kodak Research Laboratory. The beef red blood cells were washed three times in Ringer-Locke solution before being used.

Experimental procedure. Following preparation of the fluid to be perfused, the rabbit was anesthetized with 15 cc. of a 20% solution of ethyl urethane in Ringer-Locke injected intravenously. The left kidney was exposed and the renal artery cannulated. The kidney was then cleared by preliminary perfusion of oxygenated Ringer-Locke for a period never exceeding 2 minutes from a small apparatus erected over the operating table. No mechanism for the establishment of pulsatile pressure was incorporated in this apparatus. If the kidney failed to clear uniformly, for any reason, it was discarded and that of another animal used. During this initial perfusion thin sections of the right kidney were removed and fixed in Champy fluid. Upon the uniform clearing of the kidney, it, and its attached cannula, were immediately transferred to the regular perfusion apparatus.

Perfusion continued for the desired period of time. During this period the maintenance of the proper perfusion pressures was controlled and the measurement of the rate of flow of the fluid was made at 3-minute intervals.

Following the perfusion of the experimental fluid the kidney was perfused for an additional 10 minutes with 1/4000 concentration of trypan blue in Ringer-Locke fluid.

Preliminary experiments were performed in which trypan blue solutions of concentrations 1/1000, 1/2000, 1/4000 and 1/8000 were perfused for 10 minutes. The kidneys were sectioned and studied. The possibility of a toxic effect being exerted on the cells by the dye was thus tested. Even with the highest concentration used no cytological change which could be attributed to toxicity of the dye was observed. However, as a margin of safety, a concentration of 1/4000 was used in all experiments.

At the conclusion of the perfusion of the dye thin sections from four different areas of the cortex were removed and placed in Susa's and Champy's fluids. Pfühl ('31) recommends Susa as the best fixative for trypan blue. The Susa-fixed sections were cut 5 μ and the Champy-fixed sections, 2 or 3 μ. The latter sections were stained with Heidenhain's hematoxylin and counterstained with Congo red.

Perfusion was performed with each of the four fluids previously listed for periods of 15, 30, 60, 90 and 120 minutes.

The appended figures (1 to 8 inclusive) are photomicrographs of Champy-fixed proximal convoluted tubules. The figures 1 a to 8 a inclusive are sketches of the photomicrographs. The figures on plate 3 are traced microprojection drawings of Susa-fixed sections of the cortical area of the perfused kidney showing the amount of stained epithelial tissue resulting from the perfusion of the experimental fluids and the dye.

EXPERIMENTAL OBSERVATIONS

Particular attention was paid to the cells of the proximal convoluted tubules. In order that the structural changes in the perfused cells may be more readily comprehended, a brief description of the normal proximal convoluted tubule should be given. The studies of Edwards ('29) and Defrise ('32) have demonstrated segmental differentiation in the proximal convoluted tubules of fishes, and there is some evidence that such a differentiation may exist in the mammal. Thus Suzuki ('12) was led to postulate several segments in the mammalian proximal convoluted tubule. Under the circumstances, reference to the cytology of the normal proximal convoluted tubule in terms of a single picture is probably not justified. However, some point of departure had to be employed as a control to which the cytological variations in the perfused cells might be referred. Sections of the right kidney of each animal were fixed in Champy's fluid (fig. 1) and the cytological changes in the perfused left kidney are contrasted in a general way with the cytological aspects of the unperfused organ.

Trypan blue control. To observe the reaction of cells to trypan blue alone four kidneys were perfused for 10 minutes each with 1/4000 concentration of trypan blue in Ringer-Locke. Sections were fixed in Susa and studied. Practically all epithelial tissue has a faint pink coloration, the result of the penetration of the highly diffusible component of the dye. This does not signify either pathological or physiological change. Occasionally a tubule cell is seen which, although still intact in the epithelium, is stained a diffuse blue. Such a cell is regarded as having completed its life role and, being dead, would soon have been sloughed into the lumen. Cells that have been sloughed into the lumina are occasionally seen and are always stained a diffuse blue. Connective tissue cells in the interstitial spaces as well as the squamous cells of the parietal and visceral layers of Bowman's capsule show a blue coloration. The basement membrane of the tubule cells and the elastic fibers of the arterioles are selectively stained by the blue component.

Perfusion of Ringer-Locke. a. Champy-fixed tissue. Perfusion for 15 minutes induced definite pathological reactions in many cells. Considerable clumping of mitochondrial granules and filaments is evident in the infranuclear portion of these cells (fig. 2). In other cells it is apparent that the mitochondrial filaments do not extend as far into the supranuclear area as is observed in the unperfused controls. The lumina are considerably distended, the epithelium flattened, and the brush borders in some instances give evidence of erosion. Throughout the longer perfusions the progression of disintegration is apparent (fig. 3). More basal clumping of mitochondrial material, continued erosion of the brush border involving eventually the distal portion of the cells and ruptured basement membranes are all evident criteria of cellular degeneration.

b. Susa-fixed tissue. The amount of diffuse staining of tubule epithelium following 15-minute perfusion of Ringer-Locke is diagrammatically represented in figure A. Perfusion for longer periods of time causes an increase in the amount of diffuse staining as is evident in figure B which portrays the amount of staining following 60-minute perfusion and in figure C which shows the staining following 120-minute perfusion.

Perfusion of Ringer-Locke plus gelatin. a. Champy-fixed tissue. The shortest period of perfusion to which the kidneys were subjected with this fluid produced some pathological reaction (fig. 4). Basal clumping of the mitochondrial material is neither as pronounced nor as extensive as that following perfusion of the fluid without the colloid. Nevertheless evidences of disintegration are present. The brush borders occasionally appear eroded. The lumina are distended and the epithelium flatter. Longer perfusions produced greater disintegration (fig. 5). This latter disintegration is not as extensive, however, as that following perfusion of the colloid-free fluid for the same length of time. Edema, as evidenced by the extent of the intertubular spaces, is not as pronounced as that induced by perfusion of Ringer-Locke.

b. Susa-fixed tissue. That 15-minute perfusion of this fluid has had destructive properties is evident from the amount of staining demonstrated in figure D. The amount of staining following 60- and 120-minute perfusions is seen in figure E and figure F, respectively.

Perfusion of Ringer-Locke plus beef red blood cells. a. Champy fixed tissue. Fifteen-minute perfusion produced no pronounced disintegration (fig. 6). The epithelium is flatter and the lumina larger than in the normal unperfused tissue. An occasional slight sloughing of the brush borders is seen. The mitochondrial material has maintained its linear arrangement in the form of both granules and filaments. Furthermore the normal length of the granules and homogeneous filaments is still approximated. Following 30-minute perfusion some basal clumping is apparent. Practically all cells show a disintegration of the mitochondrial filaments into dispersed granules in the distal portion of the cells with linear relationship maintained only in the proximal portion. Sloughing of the brush border has progressed. Sixty-minute perfusion reveals but little change in the mitochondrial reaction from that observed following 30-minute perfusion. A semblance of linear arrangement of granules and filaments remains (fig. 7). There are many tubules which still show normal brush borders. The intertubular spaces are widened somewhat indicating an edematous condition. Ninty-minute perfusion as well as 120-minute perfusion reveal a slow progression of pathological manifestations. Occasionally erosion has involved the distal portion of cells. The majority of cells exhibit a dispersal of coarse and fine granules. Basal clumping is in evidence but not preponderantly so. A more edematous condition is apparent.

b. Susa-fixed tissue. Perfusion for 15 minutes caused no staining of the tubular epithelium (fig. G). Thirty-minute perfusion induced staining of a small amount of tissue and 60-minute perfusion (fig. H) but little more. The amount of staining resulting from 120-minute perfusion is seen in figure I.

Perfusion of Ringer-Locke plus gelatin plus beef red blood cells. a. Champy-fixed tissue. The shortest period of perfusion induced no pronounced pathological reaction. Mitochondria extend throughout the length of the cell. Occasionally a slightly frayed brush border is to be seen. Thirty-minute perfusion induced definite pathological results. Basal clumpings of mitochondrial material is evident. The majority of the brush borders show some degree of sloughing. The length of the mitochondrial filaments has been shortened (fig. 8). The longer perfusions reveal an increase in the amount of mitochondrial clumping and in the erosion of the brush borders. Following 120-minute perfusion there is little evidence of a linear arrangement of mitochondrial filaments or granules. The distal portions of a few cells are eroded and a few basement membranes are ruptured. Intertubular spaces suggest a slight edema.

b. Susa-fixed tissue. The amount of staining following 15-minute perfusion of this fluid is seen in figure J. That following 60- and 120-minute perfusion is seen in figure K and figure L, respectively.

DISCUSSION

In preliminary experiments by the writer an evaluation of a series of fluids had been attempted on the basis of mitochondrial reaction. It was believed that a dissolution of mitochondrial filaments was an index of a pathological condition. The work of Ophüls ('07), Barratt ('13), Lewis and Lewis ('15), Scott ('16) and Oliver ('16) prompted the assumption. On the other hand Takaki ('07), Kolster ('11) and Oliver and Lund ('33), in inanition, diuresis, forced feeding, dehydration and perfusion experiments, have suggested that the transformation of mitochondrial filaments into series of granules is an index of a secretory stage. Takaki, and Oliver and Lund have furthermore suggested that a close relationship exists between the mitochondrial reactions manifest during secretion and those occurring in early degeneration. Consequently any attempt to evaluate the adequacy of perfusion fluids as environmental media on the basis of mitochondrial reaction alone had to be tempered by the possibility that a sharp line of demarcation may not separate the physiological and pathological alterations of these particular organoids. The basal clumping of amorphous material associated with later degeneration stages is, however, obviously pathological. The vital dye, trypan blue, was employed with the hope of differentiating between the physiological changes and pathological changes in early degeneration.

It is known definitely that diffuse staining of cells by the dye indicates death. That a severely injured cell will also stain diffusely has been suggested (von Möllendorff, '20; Cappell, '20). Kiyono ('14) has classified the degree of cellular injury according to the type of reaction of the cells of the injured tissue to the dye. Turk's ('13) interpretation of her experiments agrees essentially with Kiyono's classification. However, it is to be observed that the initial stages in Kiyono's classification involves the disappearance of the segregated trypan blue granules which do not appear in the cytoplasm until at least 3 hours following the injection.

Obviously a 10-minute perfusion of the dye is not ample time to permit segregation of the trypan blue granules to occur. There is thus this disadvantage to the perfusion method of subjecting tissue to trypan blue or any vital dye. The short period of application does not permit detection of early degeneration. The death of a cell is a progressive phenomenon. The stages in the process may be summarily classified as follows:

1. Living—normal functional activity.
2. Reversibly moribund—injured, with recovery possible.
3. Irreversibly moribund—injured, with recovery impossible.
4. Dead.

With the injection technique it may be possible to designate approximately the stage that the cell has reached. With the perfusion method, however, the fourth stage alone can be designated unequivocally. The third stage, characterized by severe injury with diffuse staining, might likewise be included since most workers believe that the dye will stain a severely injured cell. However the second stage and its gradation into the third stage can no more be detected by the perfusion of the dye than it can by a study of the mitochondrial reaction. Hence the perfused dye method has not completely surmounted the difficulty with the mitochondrial observations as mentioned above but it has its merits in that it corroborates conclusions drawn from mitochondrial reactions and furthermore permits a more rapid and more thorough appraisal of severe cell injury and death than does the study of mitochondria.

It is quite clear that none of the perfusion fluids as used in these experiments is completely adequate to maintain the normal functional condition of the kidney for a prolonged period of time. Fluids without oxygen carriers are inadequate for even the shortest period of perfusion reported. The fluids with blood cells are seemingly adequate for the shorter perfusions but they are not completely adequate as environmental media as shown by the increased pathological effects after 30-minute perfusion.

In all probability fluids of this type are much too elementary for prolonged perfusion. A need for neutral fats or lipoidal material, hormones, vitamins and amino acids might be suggested. It is possible that some protein other than gelatin would be more efficient provided it could be easily obtained in sufficient degree of purity. It is quite probable that the efficiency of the colloid depends on some characteristic other than or in addition to its simple colloidal properties. The possibility that perfusion in an open system may wash out essential cellular constituents as Clark ('13) has shown in the perfusion of the frog heart with Ringer-Locke solution presents itself. Carrel ('13) and Ebeling ('19) have found that the maintenance of life in tissue growing in vitro is possible only through the addition of expressed tissue extract to the plasma culture. The element or elements in the extract responsible for the promotion of life have not been found. Carrel and Lindbergh ('35) in their study of the culture of whole organs specify as one of the ultimate purposes of their experiments "the isolation of substances essential to the growth, differentiation and functional activity of the glands." Apparently this is the most urgent task facing the student who uses the perfusion method.

SUMMARY AND CONCLUSIONS

A method of evaluating, cytologically, the adequacy of synthetic fluids to maintain perfused tissue in the normal condition has been found. The method involves the perfusion of a vital dye, trypan blue, immediately following perfusion of the fluid in question. Trypan blue stains tissue that has undergone severe pathological changes or death.

The method has been tested by observing the reaction of mammalian kidney tissue to a series of four perfusion fluids graded with respect to their physiological adequacy. These fluids were: Ringer-Locke; Ringer-Locke plus gelatin; Ringer-Locke plus beef red blood cells; Ringer-Locke plus gelatin plus beef red blood cells.

The efficacy of the dye used in this way was furthermore tested by comparing the amount of staining with the mitochondrial reaction to these fluids.

The most perfect fluids, theoretically, were completely adequate only for short periods (15 minutes) of perfusion.

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PLATE 1

EXPLANATION OF FIGURES

1 Section of unperfused proximal convoluted tubule showing the brush border and mitochondria (granular and filamentous). Champy; Heidenhain. × 2125.

1 a Sketch of figure 1.

2 Section of proximal convoluted tubule following 15-minute perfusion of Ringer-Locke. Observe the lowered epithelium as compared with that shown in figure 1. Clumping of mitochondria is evident. Champy; Heidenhain. × 2125.

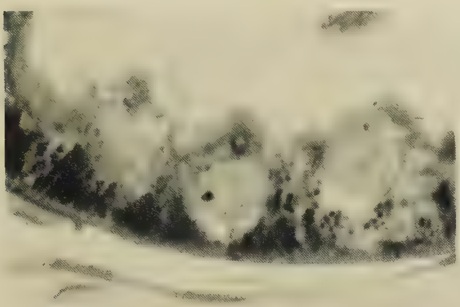
2 a Sketch of figure 2.

3 Section of proximal convoluted tubule following 90-minute perfusion of Ringer-Locke. Observe the lowered epithelium as compared with that shown in figure 2. Fairly normal brush border is evident. Clumping of mitochondria with complete loss of linear arrangement may be discerned. Champy; Heidenhain. × 2125.

3 a Sketch of figure 3.

4 Section of proximal convoluted tubule following 15-minute perfusion of Ringer-Locke plus gelatin. Observe the normal height of the epithelium, slightly eroded brush border, and linear arrangement of mitochondrial granules and filaments. Slight evidence of mitochondrial clumping. Champy; Heidenhain. × 2125.

4 a Sketch of figure 4.



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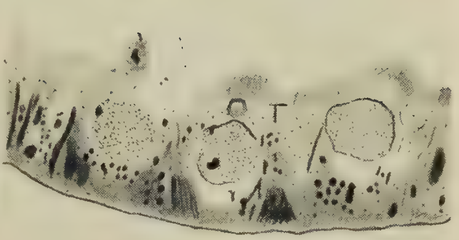

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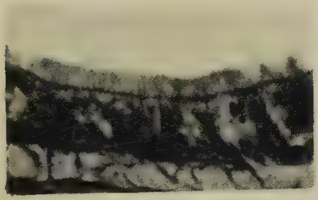

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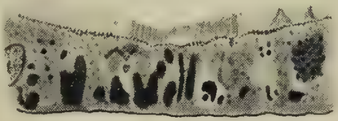

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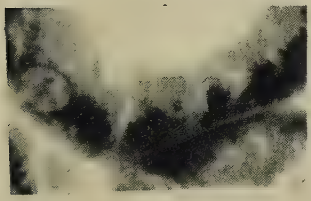

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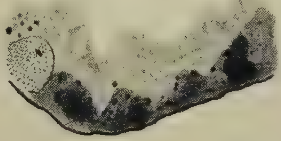

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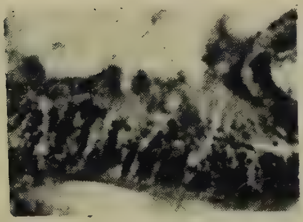

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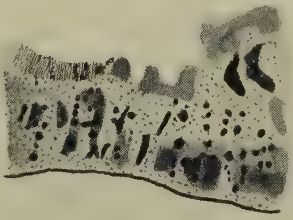

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PLATE 2

EXPLANATION OF FIGURES

5 Section of proximal convoluted tubule following 120-minute perfusion of Ringer-Locke plus gelatin. Observe the lowered epithelium as compared with figures 1 and 4. Slight erosion of the brush border. Clumping of mitochondria is apparent. Champy; Heidenhain. × 2125.

5 a Sketch of figure 5.

6 Section of proximal convoluted tubule following 15-minute perfusion of Ringer-Locke plus beef red blood cells. Observe the normal height of the epithelium, the slightly eroded brush border, and the filamentous mitochondria. No apparent clumping. Champy; Heidenhain. × 2125.

6 a Sketch of figure 6.

7 Section of proximal convoluted tubule following 30-minute perfusion of Ringer-Locke plus beef red blood cells. Observe the lowered epithelium as compared with that shown in figure 6. Linear arrangement of mitochondrial material maintained in the proximal portion of the cells. Champy; Heidenhain. × 2125.

7 a Sketch of figure 7.

8 Section of proximal convoluted tubule following 30-minute perfusion of Ringer-Locke plus gelatin plus beef red blood cells. Observe the height of the epithelium as compared with that shown in figure 7. Brush border is somewhat eroded, mitochondrial filaments shortened. Some evidence of clumping of mitochondria. Champy; Heidenhain. × 2125.

8 a Sketch of figure 8.

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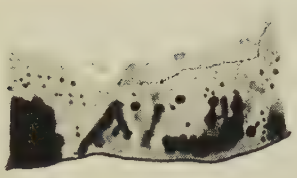

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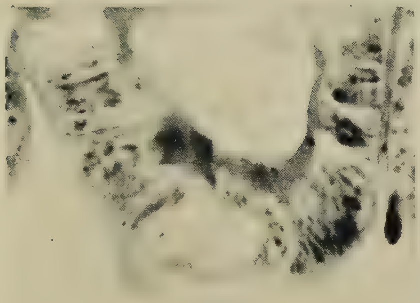

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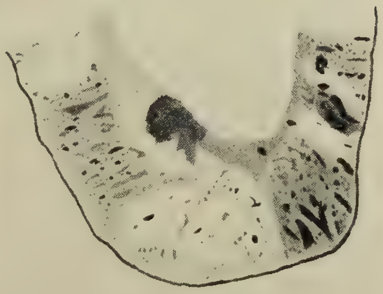

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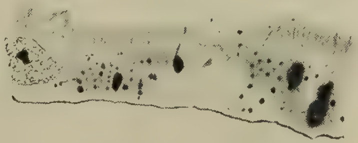

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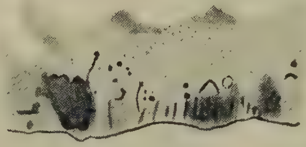

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PLATE 3

EXPLANATION OF FIGURES

Reduced projection drawings of sections of renal cortex showing relative amounts of stained tissue following perfusion of the various fluids listed below with subsequent 10-minute perfusion of trypan blue in Ringer-Locke (1/4000).

Ringer-Locke

	Length of perfusion
A	15 minutes
B	60 minutes
C	120 minutes

Ringer-Locke plus gelatin

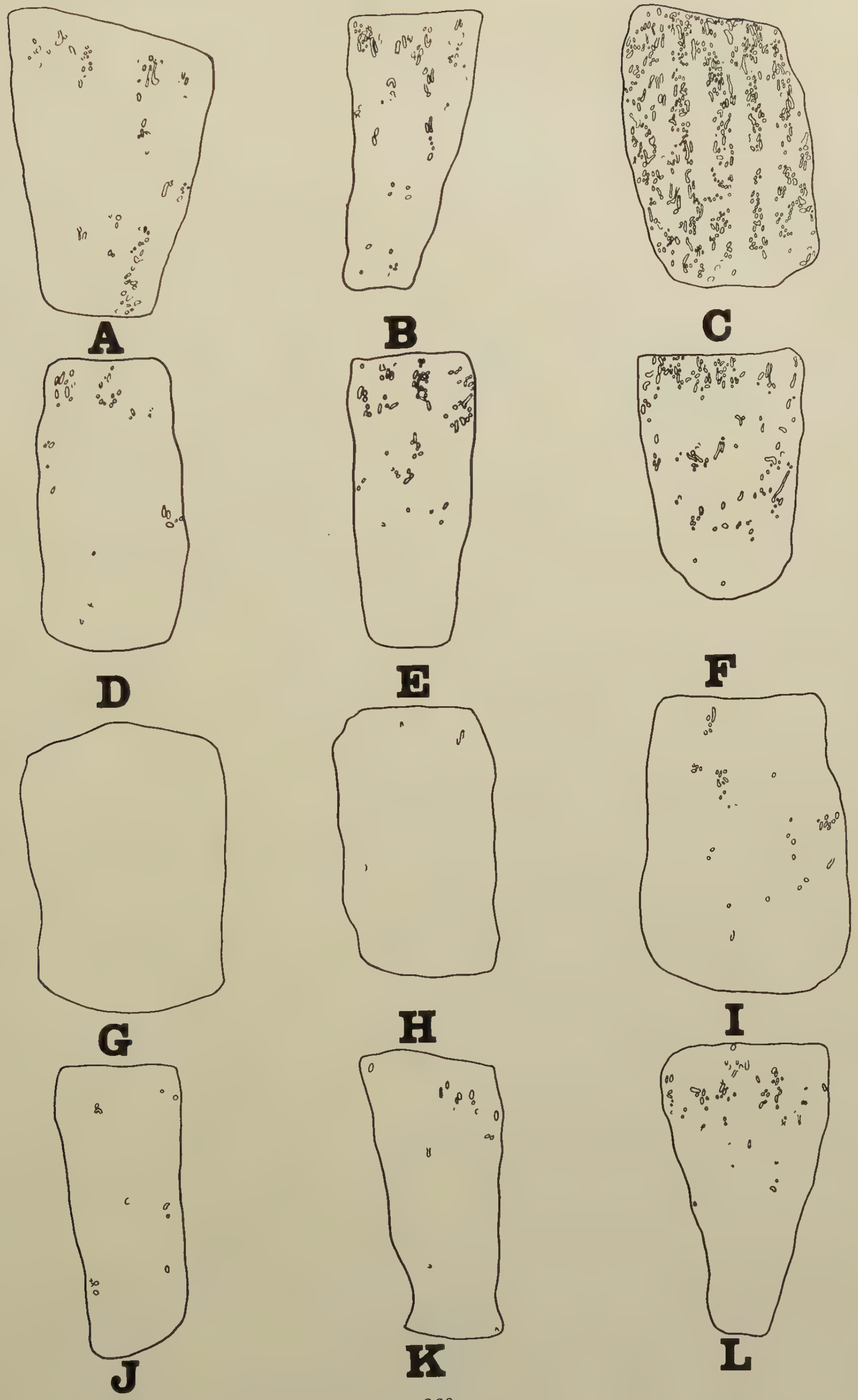
	Length of perfusion
D	15 minutes
E	60 minutes
F	120 minutes

Ringer-Locke plus beef red blood cells

	Length of perfusion
G	15 minutes
H	60 minutes
I	120 minutes

Ringer-Locke plus gelatin plus beef red blood cells

	Length of perfusion
J	15 minutes
K	60 minutes
L	120 minutes


A
B
C
D
E
F
G
H
I
J
K
L

NEW BOOKS AND PUBLICATIONS

ILLUSTRATIONS OF REGIONAL ANATOMY, by E. B. Jamieson. 1936. Section VI. Upper Limb. 42 plates. $6\frac{1}{2} \times 8\frac{1}{2}$ inches. Loose leaf binding of cardboard. Price, $2.00. William Wood and Company, Baltimore.

ILLUSTRATIONS OF REGIONAL ANATOMY, by E. B. Jamieson. 1936. Section VII. Lower Limb. 52 plates. $6\frac{1}{2} \times 8\frac{1}{2}$ inches. Loose leaf binding of cardboard. Price, $3.00. William Wood and Company, Baltimore.

These two sections continue an excellent series of illustrations of regional anatomy. The plates are arranged in a sequence suitable for reference during the progressive steps of dissection. The pictures are somewhat diagrammatic, aiming rather for clear showing of structures and relations than for depth and realism. All the illustrations are bound into volumes by an ingenious arrangement that allows any plate to be removed or inserted readily. Each plate is complete with explanatory legends.

BONES. A STUDY OF THE DEVELOPMENT AND STRUCTURE OF THE VERTEBRATE SKELETON, by P. D. F. Murray. 1936. Size, $5\frac{1}{4} \times 7\frac{3}{4}$ inches. Pages x + 203. 45 illustrations. Index. List of references. Cloth. Price, $2.50. The Macmillan Company, New York.

This book discusses briefly two groups of factors influencing the structure of bones: morphogenic factors intrinsic in the elements and outside factors mostly of a mechanical nature. It gives a fair statement of the problem as it is today and suggests probable development and profitable lines of research. As stated in the preface ''The main mass of the book is in the first four chapters, which deal with the embryonic and postembryonic development and with the structure of the normal and modified bony skeleton considered in relation to the function it fulfills. In a brief fifth chapter cartilage is dealt with from the same point of view. The sixth is devoted to a consideration of the mechanisms by which mechanical forces find expression in the architecture of bone, and the seventh is a very brief summary.''

THE HARVEY LECTURES. Delivered under the auspices of The Harvey Society of New York. 1935–1936. Series XXXI. Size, $5\frac{1}{2} \times 8\frac{1}{4}$ inches. 225 pages. Lists of officers and members. List of references after each article. Illustrated. Cloth. Price, $4.00. The Williams and Wilkins Company, Baltimore.

The usual high standard of the Harvey Lectures has been maintained in this group of eight distinguished scientists each of whom is preeminent in his own field. The titles and authors are as follows: Proteins and Proteolytic Enzymes, by Max Bergmann; The Significance of Chimpanzee-Culture for Biological Research, by Robert M. Yerkes; The Virus Tumors and the Tumor Problem, by Peyton Rous; Relations between the Parathyroids, the Hypophysis and the Pancreas, by B. A. Houssay; The Interrelation of Cerebrum and Cerebellum in the Regulation of Somatic and Autonomic Functions, by John Farquhar Fulton; The Influenzas of Swine and Man, by Richard E. Shope; Malignant Cells, by Warren H. Lewis; The Physiology of the Bronchial Vascular System, by I. de Burgh Daly.

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D. H. Tennent	*General Embryology*
M. H. Jacobs	*General Physiology*

Representative on the Executive Committee of the Commission for the Standardization of Biological Stains

S. I. Kornhauser

Managing Editor of the Journal of Morphology

To serve until December, 1941 .... C. E. McClung

Members of Editorial Board of the Journal of Morphology

To serve until December, 1937
D. H. Tennent L. C. Dunn C. G. Hartman
To serve until December, 1938
Sally Hughes-Schrader E. E. Just D. H. Wenrich
To serve until December, 1939
Leigh Hoadley W. A. Kepner J. Percy Moore

PROCEEDINGS OF THE THIRTY-FOURTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF ZOÖLOGISTS

ATLANTIC CITY, N. J., DECEMBER 29–31, 1936

PROGRAM

The Society met with the American Association for the Advancement of Science. The sessions of the Society were held in the Municipal Auditorium of Atlantic City.

In addition to the abstracts printed in the December supplement of this journal the Society by vote authorized the printing of the following late abstracts:

231. THE CHEMICAL IDENTITY OF THE ENDOPLASMIC CRYSTALS OF PARAMECIUM. A. W. Bernheimer, Temple University. (Introduced by A. A. Schaeffer.)

Optical tests on the crystals of Paramecium multimicronucleatum show that their refractive indices and crystal system correspond with those of melamine, the amide of cyanuric acid. The crystals agree with melamine in other properties among which are their solubility in about twenty reagents and their tendency to sublime when cautiously heated.

From the large amount of nitrogen in the melamine molecule, it seems probable that the chief nitrogenous product of metabolism is neither urea nor uric acid. The crystals of paramecium may function as a substance arising through a process of rendering nitrogenous compounds non-toxic. The molecular structure of melamine indicates that it might form through the cleavage of some amino acid.

232. THE EFFECT OF ULTRA-VIOLET LIGHT UPON MATURATION IN EGGS OF URECHIS CAUPO. H. Y. Chase, Howard University. (Introduced by E. E. Just.)

Eggs of Urechis caupo, Fisher and MacGinitie, exposed to ultra-violet light and fertilized, developed with or without complete maturation depending upon the length of exposure. In the experiments whose controls showed 99% to 100% polar body extrusion, eggs were irradiated for 30, 35, 40, 45, 50, 55 and 60 seconds. Accurate counts of polar bodies were made for 24,000 eggs at each exposure. It was found that the percentage of first and second polar bodies extruded varies inversely with the length of exposure. Exposures of 30 seconds gave 91.3% first and 88.9% second polar bodies while exposures of 60 seconds gave 2.3% first and 0.68% second polar bodies.

These experiments show that irradiation of unfertilized eggs of Urechis caupo affects the normal maturation process.

233. TOTIPOTENCY OF REGENERATING MESENCHYME OF ADULT AMPHIBIANS. Oscar E. Schotté, Amherst College.

An attempt has been made to test the ability of regenerating tissues of adult Triturus pyrogaster, T. viridescens, old larvae of Amblystoma punctatum and of old tadpoles of R. catesbiana and R. pipiens in submitting them to the influence of the eyeball previously deprived of its lens. After removal of the lens in an adult urodele (in other experiments cornea and lens are removed) or in an old tadpole (R. pipiens chiefly), a several days old blastema of leg or tail is placed on top of the open pupil with its mesodermal part directed toward the eye chamber. After a few days the transformation of the regenerating mesenchyme into a new formation with all the morphological characteristics of a lens has been observed many times. In several cases, serial sections show the new lens differentiating itself in the midst of the transplanted material or still attached to it by a bridge of tissues. In other experiments new cornea and also retina have been observed to arise from the transplanted blastema.

Histological study allows little doubt that the mesenchyme of the regenerate alone is being thus induced into embryonic organs, while the ectodermal part of the regenerate remains unaffected.

From the above, it can be inferred that regenerating mesodermal tissue of adult amphibians is totipotent as it can manifest its capacity for a new embryonic redifferentiation similar to that demonstration by the classical experiments of Spemann, Harrison and their schools on early embryonic stages.

The total number of papers listed to be read was 120 (including the symposium and special invitation programs), presented by demonstration 28 and read by title, 91.

The biologists' smoker was held Tuesday evening in the Renaissance Room of the Hotel Ambassador with an attendance of probably about 800. The annual dinner was also held in the Renaissance Room on Wednesday evening with 270 attending. The annual address was given by the Vice-President of Section F, Dr. Ross G. Harrison of Yale University.

BUSINESS MEETING

The thirty-fourth annual business meeting was opened at 12.10 P.M. Tuesday, December 30th, with President Allee presiding.

The following business was transacted:

1. The minutes of the last meeting having been previously distributed to the members in the January, 1936, supplement to The Anatomical Record were declared approved as published.

2. By vote of the Society the secretary was authorized to publish late abstracts in the January, 1937, supplement to The Anatomical Record.

3. *Report of the secretary.*

a. Membership. The membership lists will show the following changes if the Society elests members as recommended by the executive committee:

Deaths	6	4 active members 2 associates	Reinstated	7
Resignations	5	2 active members 3 associates	New active members	20
Dropped	9	6 active members 3 associates	New associates	42
	20			69

Total gain in enrollment	49
Enrollment in last report	718
Total enrollment this report	767
Associate members advanced to active	17

Summary of enrollment

Life members	5
Active members	579
Associate members	183
	767

b. Result of ballot taken during the current year in regard to frequency of meeting with the A.A.A.S.

	First choice	*Second choice*	*Third choice*	*Fourth choice*
All meetings with A.A.A.S.	157	31	24	66
Occasional meetings independent of A.A.A.S.	113	97	58	13
Alternate meetings with A.A.A.S.	40	95	106	1
Occasional meetings with A.A.A.S.	73	37	38	73
No meetings with A.A.A.S.	8			

Result of ballot upon summer meeting at Yellowstone Park.

For	*Against*	*Blank or indifferent*	*Total ballots cast*
136	98	164	398

It was voted to accept the report and instruct the secretary to designate the founders of the Society in the list of members.

(*Note*: As the original records, which are filed at the Marine Biological Laboratory, cannot be consulted until after the manuscript for the January, 1937, lists goes to press this change will first appear in the 1938 lists.)

4. *Report of the treasurer.*

The following report was presented by the treasurer:

Report of the Treasurer for the year 1936

Credits:			
Balance on hand December 30, 1935, when accounts were last audited	$ 965.82		
Receipts from dues, 1936	2530.51		
From treasurer, Genetics Society	25.00		$3521.33
Expenditures:			
Annual meeting (Princeton)		$ 125.00	
Secretary's expenses:			
Stenographer	34.50		
Printing and stationery	52.80		
Postage and express	52.73		
Telephone and telegraph	13.89		
Expenses at scientific meetings	44.38	198.30	
Treasurer's expenses:			
Postage	31.00		
Stenographer	8.00		
Printing	38.14		
Supplies	2.28	79.42	
Wistar Institute:			
Proceedings and list of members	145.64		
Abstracts, etc.	492.51	638.15	
Biological abstracts		1132.00	
Two checks returned (Green, Hess)		8.00	
Bank charges		8.18	
		2189.05	
Balance on hand December 22, 1936, in Hayes National Bank:			
Checking account less outstanding check	$ 332.28		
Savings account	1000.00	1332.28	
		$3521.33	$3521.33

Increase in funds since December 30, 1935, amounts to $366.46

5. *Report of the auditing committee* (W. C. Cole, Elmer G. Butler).

Dr. W. C. Cole, chairman, reported that the report of the treasurer had been examined and found to be correct.

It was voted to accept and approve the reports of the treasurer and of the auditing committee.

6. *Report of representative on Commission for Standardization of Biological Stains.*

The following report was read and approved:

> As representative of the zoölogists on the Commission for Standardization of Biological Dyes, I wish to report that during the year my chief activities have been concerned with the biological testing of the stains listed as follows: eosin, carmine, congo red, azocarmine G, Jenner, and tetrachrome.
>
> Your representative was instrumental in getting the National Aniline Company to manufacture azocarmine G for use in the azan stain of Heidenhain which is used so widely now. The American azocarmine has proved quite as satisfactory as that which was formerly imported.
>
> I wish also to call your attention to two activities of the commission. During the past year Stain Technology has issued a considerable number of abstracts of papers on technical microscopical subjects, and these abstracts are given in enough detail so that one can follow the procedure without, in general, consulting the original article. There has been a new edition published of the little book, Biological Stains, this being the third edition in a little over 10 years, showing that the book is being well received by the scientific world.
>
> Reports have come to your representative concerning failure of several American dyes to come up to expectations, but in each case it was ascertained that each sample did not bear the certification label of the commission and it was impossible to run down the cause of failure.
>
> S. I. KORNHAUSER.

7. *Report of committee on Educational Tests* (D. E. Minnich, W. C. Allee, R. A. Budington).

Dr. D. E. Minnich reported that the committee found that a thorough survey would be necessary and that the committee had applied to the American Council on Education for funds for this purpose but no arrangement has been completed. The report was accepted.

8. Franklin Davis Barker, Charles Velmar Green, Reginald Gordon Harris and Charles E. Johnson, active members of the Society, and Paul Chesley and Clifford H. Alvey, associate

members of the Society, died during the past year. The following resolutions were adopted by a rising vote of the Society and the secretary was instructed to send copies to the next of kin and to publish the resolutions in the annual report of the meetings.

Active members

FRANKLIN DAVIS BARKER

Franklin Davis Barker was born on September 16, 1877, at Ottawa, Kansas. He attended Ottawa College, from which he received the A.B. degree in 1898 and the A.M. degree in 1900. Later he went to the University of Nebraska and received a Ph.D. degree in 1910. While in Nebraska he worked with Prof. H. B. Ward and developed a lasting interest in parasitology, He became a member of the faculty at the University of Nebraska in 1903. His interest in premedical education was marked and when in 1910 he became professor of zoölogy, he was appointed director of premedical studies. He remained at Nebraska until 1926 when he was called to Northwestern University as head of the zoölogy department and occupied this position until his death on July 10, 1936. His regime at Northwestern University was marked by an extensive and substantial growth in the department of zoölogy.

From 1905 to 1907 Doctor Barker was secretary of the Nebraska Academy of Science and for a number of years he was an editor of the Journal of Parasitology.

Doctor Barker's own research was distinguished for its carefulness and accuracy and he had the happy faculty of drawing out those same qualities in the work of his graduate students. A considerable number of young men have begun their careers in parasitology with him and have since made substantial progress. His teaching was characterized by patience and a sympathetic understanding of the problems of his students. His influence will live long in the lives of his students and in his chosen field of research.

C. L. TURNER.

CHARLES VELMAR GREEN

The death of Dr. Charles Velmar Green occurred on April 17, 1936 from accidental drowning. Doctor Green had just passed his thirty-fourth birthday. He was of a quiet and studious disposition and had in the all too brief span of his efforts as a research worker contributed notably to our knowledge of the genetics of growth in rodents. He had been able to establish the fact that there was association in inheritance between certain genes for color formation and other genes for size in mice. To this problem, which had been under investigation in several laboratories for many years, he brought order and information which more nearly approached a solution than had that contributed by any of his contemporary or previous workers. His interest in genetics was broad and constructive. He was versatile and extremely energetic.

He received his bachelor's degree at Michigan State College and his Ph.D. degree at the University of Michigan. Since its foundation in 1929 he had been a research associate at the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. His part in the work of that institution was so important and vital that his associates will find it difficult if not impossible to fill his place.

C. C. LITTLE.

REGINALD GORDON HARRIS

The death of Reginald Gordon Harris on January 7, 1936, at the age of 37, deprived us of one of our promising members. Doctor Harris through his work as director of the Biological Laboratory at Cold Spring Harbor had already attained a conspicuous position in the scientific world. He had created there what promises to be one of the leading research laboratories in biophysics, and, by the inauguration of yearly symposia, had made it one of the centers for exchange and dissemination of ideas concerning the relation of the basic sciences to biology. His gracious personality and hearty enthusiasm fitted him for his position as organizer and leader. All of us who have known him intimately as colleague and friend are sensible of a great loss at his passing.

Doctor Harris was a graduate of Brown University in 1918, Ph.D. in 1924, and an instructor in biology 1920–1921. He studied in Professor Caullery's Laboratory in France. He was a member of the Cornell entomological expedition to South America, 1919–1920. He had travelled widely in Northern Africa and Central America. His scientific interests varied from paedogenesis in Diptera to the ethnology of the white Indians in Darien.

J. WALTER WILSON.

CHARLES E. JOHNSON

Charles E. Johnson was born in Oslo, Norway, on April 24, 1880. He came to this country with his parents at the age of two, and the family settled in the town of Warren, Minnesota.

His educational training consisted of attendance at the Warren High School, and the University of Minnesota, A.B., 1906; A.M., 1907; Ph.D., 1912. During the period of his graduate study and immediately afterward, he was intensely interested in wild life and made extensive travels on foot, photographing and recording his studies on wild life as he saw it.

His early teaching was at the State Universities of Minnesota and Kansas. He came to the New York State College of Forestry in 1923 as professor of forest zoölogy, and in 1926 he was appointed director of the Roosevelt Wild Life Station. Professor Johnson's interests were threefold, teaching, research, and the setting of a high standard for the numerous contributions from the Roosevelt Wild Life Station. As a teacher his intimate knowledge of animals as they exist in nature stimulated his students to undertake critical field studies. His early training in embryology and anatomy equipped him with an admirable foundation for his studies in vertebrate ecology. Notable among these are his papers on the beaver in the Adirondacks and the muskrat in New York State.

The scope and contents of the bulletins of the Roosevelt Wild Life Station are shown on pages 10 to 13 of the Bulletin of the New York State College of Forestry, vol. 8, no. 2A. Each of these bulletins represented his meticulous supervision, the facts being checked over and over. The superficial studies of an earlier date had brought natural history into disrepute, and Doctor Johnson has done much to restore this very important phase of zoölogical investigation to a high scientific plane.

In connection with this work, there has been built up a library of some 10,000 pieces of literature which emphasize natural history studies. This collection has been made primarily through Professor Johnson's efforts to develop a wide exchange list. The bulletins of the Roosevelt Wild Life Station and the large library accumulated by him will furnish a permanent and valuable legacy for Professor Johnson's colleagues in zoölogy and for future students of natural history.

W. M. SMALLWOOD.

Associate members

PAUL CHESLEY

Paul Chesley was born in West Newton, Massachusetts, July 10, 1906, the son of Harry and Emma Putnam Chesley. He was graduated from Bates College in 1929 and in the autumn of that year matriculated as a candidate for the doctorate in Columbia University, receiving at the same time an appointment as assistant in Zoölogy in Seth Low Junior College. In 1931 he was advanced to an instructorship in the same college and held this appointment until his death. He obtained the degree of doctor of philosophy in 1934.

Doctor Chesley's interest in zoölogy declared itself during his college course and during his junior and senior years he served as assistant in biology. At Columbia his interests became focussed on the embryological events through which the mutant characters of the adult are realized, and he began, in 1930, a genetical and embryological analysis of the short-tail (brachyury) mutant of the house mouse. His two published papers on this subject (Proc. Soc. Exp. Biol. and Med., 1932, 29: 437–438 and J. Exp. Zoöl., 1935, 70: 429–459) provided the first embryological description of the effects of a mammalian lethal on early development and showed that the mutation had altered certain fundamental structures of the embryo, and produced widespread changes in the central nervous system and skeleton. He began in 1930 the analysis of several tailless mutations of the house mouse in which the above lethal proved to be balanced by other lethals which brought about the death of another class of embryos at even earlier stages, and he was actively engaged in this work at the time of his death. A portion of his results have been published (Genetics, 1936, 21: 525–536).

His work throughout was marked by extreme thoroughness and care, guided by an unusual perspective of the interrelations of genetics and embryology. He was ever a severe critic of his own work and reluctant to publish results which fell short of his own criteria of completeness or conclusiveness. He consequently left unpublished considerable valuable material on several problems related to the above.

In person Doctor Chesley was to a degree reticent and modest, but these qualities failed to mask his great ability. His death removes a young scientist and teacher of great promise and one whose character has made a lasting impression on his fellows and his students.

Doctor Chesley died in New York, February 12, 1936.

L. C. Dunn.

CLIFFORD H. ALVEY

Clifford H. Alvey, B.S., Huron College, Huron, South Dakota, Ph.D., New York University, 1933, died after a brief illness—or should we say, after ten years of illness—September 10, 1935. Professor Alvey was born July 12, 1908. When a junior in Huron College, he suffered an attack of osteomyelitis—as a result of which he lost his right leg. Nevertheless, he completed his college course, and proceeded to New York University where, as a graduate student and assistant in biology, he made a large place for himself in the hearts of his colleagues. In addition to his teaching and research, he rendered a noteworthy service to the university as treasurer and steward of the Faculty Club. In 1934–1935 he came to Purdue University with the rank of instructor in the School of Science, but such was the distinction of his record as teacher, investigator, and director of research that, at the close of his first year he was promoted to the rank of assistant professor of parasitology. He spent the summer of 1935 at the Marine Biological Laboratory at Woods Hole, Mass., directing the work of students whom he had brought with him at the same time completed two papers of his own. He left Woods Hole on August 29th in apparent good health, and spent the weekend in New York, on his way to Purdue. He arrived with a severe cold, that developed into pneumonia, aggravated by a recurrence of osteomyelitis and the end came before he was able to fully realize that he should never again have the cherished opportunity to return to his laboratory.

Howard E. Enders.

9. *Election of members.*

Upon recommendation of the executive committee and by motion duly made and carried the following members were elected by the secretary casting one ballot in their favor:

ACTIVE MEMBERS

(Formerly Associate Members)

Breneman, Wm. Raymond
Cameron, J. A.
Clausen, Harry John
Doyle, W. L.
Etkin, Wm.
Evans, Titus Carr
Hardesty, Mary
Hile, Ralph
Morgan, Wm. Pitt
Pearson, N. E.
Price, Dorothy
Rugh, Roberts
Shaver, J. M.
Stone, R. G.
Strandskov, H. H.
Van Cleave, Charles D.
Watanabe, Y.

ACTIVE MEMBERS

(Not formerly Associate Members)

Beadle, George Wells
Coonfield, Benjamin Randolph
Evans, Llewellyn Thomas
Fraps, Richard M.
Irwin, Malcolm Robert
Johnson, Willis Hugh
Kinsey, Alfred C.
Le Veque, Norma
Lynch, Ruth Stocking
Marsland, Douglas A.
Miller, Alden Holmes
Myers, George Sprague
Puckett, William Olin
Rulon, Olin
Ruth, Elbert Bresee
Schmitt, Francis Otto
Scott, Gordon Hatler
Snell, George D.
Twitty, Victor Chandler
Wolf, Ernst

ASSOCIATE MEMBERS

Abramowitz, Alexander A.
Alderman, Alton Lovell
Bailey, Percy Laurance, Jr.
Bennett, Harry Jackson
Breland, Osmond P.
Buck, John Bonner
Bugbee, Robert E.
Carpenter, Esther
Child, George Percy
David, Paul R.
Denny M.
Eakin, Richard Marshall
Fennell, Richard Adams
Foster, Kendall Wilson
Gier, Herschell T.
Gluecksohn-Schoenheimer, Salome
Goldsmith, Eli D.
Holt, Vesta
Ingle, Lester
Janes, Ralph Gordon
Johnson, Maynard Stickney
Johnson, Mychyle Wellington
Kopenhaver, Mary Elizabeth
Lloyd, Harriet Exline
Loosanoff, Victor L.
Lumer, Hyman
Myers, Raymond J.
Nahm, Laura Julia
Nigrelli, Ross F.
Pierce, Madelene Evans
Pomerat, Charles Marc
Rankin, J. S.
Robertson, Charles William
Sawyer, Elizabeth Louise
Sumner, Ruth
Tasker, R. C.
Walker, Paul Andrew
Warbritton, Virgene
Wichterman, Ralph
Woodside, Gilbert Llewellyn
Worley, Leonard George
Zahl, Paul Arthur

A late application for membership was referred to the executive committee for recommendation in 1937.

10. *Report of executive committee.*

The report of the committee was as follows:

a. Nine members have been dropped for non-payment of dues for past 2 years in accordance with by-laws, and there have been five resignations.

b. Candidates were nominated for election to membership as listed under item 9 above.

c. The treasurer has been authorized in accordance with vote of Society last year to make a payment of an honorarium of $200 to secretary for year 1937.

d. The committee recommends the support of Biological Abstracts for 1937 on same plan as for 1936, i.e., $2.00 from each membership fee of $4.00 paid to Biological Abstracts.

e. President of Society was requested to appoint representatives of the Society to Council of the Union of American Biological Societies in the absence of the elected representatives.

The report of the executive committee was accepted and its recommendations adopted.

A motion was made and passed by unanimous vote that Dr. Edmund B. Wilson be elected an honorary member of the Society and that the executive committee be instructed to see that the necessary steps should be taken to provide for a class of honorary members.

11. *Report of nominating committee and election of officers.* The committee (Ulric Dahlgren, H. V. Neal, Mary Hogue) presented the following report.

President, 1 year, F. L. HISAW.
Vice President, 1 year, HELEN DEAN KING.
Secretary, 3 years, ELMER BUTLER.
Executive Committeeman, 5 years, W. C. ALLEE.
Society Representatives on A.A.A.S. Council, 1 year, D. E. MINNICH, B. H. WILLIER.
Society Representatives on Council of Union of American Biological Societies, 1 year, H. E. JORDAN, H. B. GOODRICH.
Society Representatives on Advisory Editorial Board of the Biological Abstracts, H. S. PRATT, G. K. NOBLE, D. H. TENNENT, M. H. JACOBS.
Representative of the Executive Committee of the Commission for Standardization of Biological Stains, S. I. KORNHAUSER.
Managing Editor Journal of Morphology, 5 years, C. E. MCCLUNG.
Associate Editors, 3 years, LEIGH HOADLEY, W. A. KEPNER, J. PERCY MOORE.

Motion was made and passed that the secretory be instructed to cast one ballot in favor of the slate submitted by the nominating committee.

12. The president announced his appointment of the nominating committee for 1938 as follows: Caswell Grave (chairman), Phineas Whiting, C. V. Taylor.

13. *Miscellaneous business.*

The following resolution was adopted:

Resolution recommending the appropriation of adequate funds for the maintenance and growth of the Army Medical Library's book collection and index-catalogue

The American Society of Zoölogists with 718 members, in its thirty-fourth annual business meeting held at Atlantic City, New Jersey, December 30, 1936, notes with pleasure the appearance of volume one of the Fourth Series of the *Index-catalogue* of the Library of the Surgeon-General's Office, United States Army (Army Medical Library).

After a delay of three years during which no volumes of this *Catalogue* were printed, the appearance of this first volume of the Fourth Series gives renewed assurance of the continuation of this publication, which, together with the Army Medical Library, is considered one of the outstanding contributions which our country and its Government have made to medical knowledge, and

WHEREAS, The value and usefulness of the *Index-catalogue* is dependent upon the completeness of the files of medical publications contained in the Library of the Surgeon-General's Office—a public, national, medical library, the greatest in the world, serving in its present form of administration with satisfaction the medical profession and the bio-medical libraries of our country, and

WHEREAS, In recent years the annual appropriation of the Congress has been wholly inadequate to provide sufficient funds to acquire the current medical books and periodicals issued throughout the world, so that they might be available for use throughout the country and for inclusion in the *Index-catalogue,*

THEREFORE BE IT RESOLVED, That the American Society of Zoölogists urges the Congress to appropriate annually to the Library of the Surgeon-General's Office an adequate sum for current medical books and periodicals and for the purchase of back publications lost during those recent years when the amount granted was grossly inadequate, thus depreciating the completeness and usefulness of the Library's collection; and an additional sufficient sum annually, for as many years as may be required, in order to make for the greatest possible completeness of the collection and its *Catalogue;* and

BE IT FURTHER RESOLVED, That a sum should be appropriated annually to defray the cost of printing regularly each year not less than one volume of the *Index-catalogue,* and

BE IT FURTHER RESOLVED, That a copy of these resolutions be spread upon the minutes of the annual meeting of this Society and sent to the President of the United States, the presiding officer of both houses of Congress, the Secretary of War, and the Surgeon-General of the Army.

It was resolved that the Society go on record as opposing the wholesale destruction of animal life involved in the 'anti-vermin' campaigns and that the secretary be instructed to give this resolution as much publicity as possible.

A motion was made and passed giving a vote of thanks to the retiring secretary for his services to the Society during the past 3 years.

Meeting adjourned.

H. B. Goodrich,
Secretary.

CONSTITUTION AND BY-LAWS

No change was made in the constitution and by-laws. They have therefore not been reprinted and will be found in the supplement to The Anatomical Record for December 25, 1936, Vol. 67, No. 1.

LIFE AND ACTIVE MEMBERS

(Life members indicated by an asterisk)
(Mailing address is indicated by italics)

Ackert, James Edward, A.B., A.M., Ph.D. (Illinois), Professor of Zoölogy, Parasitologist Agr. Exp. Station; *Kansas State College, Manhattan, Kan.*

Adams, Amy Elizabeth, B.A. (Mt. Holyoke), M.A. (Columbia), Ph.D. (Yale), Professor of Zoölogy, *Mount Holyoke College, South Hadley, Mass.*

Adams, Leverett Allen, A.B., A.M. (Kansas), Ph.D. (Columbia), Assistant Professor of Zoölogy, University of Illinois, *406 Nevada St., Urbana, Illinois.*

Adamstone, Frank Bolton, B.A., M.A., Ph.D. (Toronto), Associate in Zoölogy, *University of Illinois, Urbana, Ill.*

Adelmann, Howard Bernhardt, A.B., A.M., Ph.D. (Cornell), Assistant Professor in Histology and Embryology, *Stimson Hall, Cornell University, Ithaca, N. Y.*

Adolph, Edward Frederick, A.B., Ph.D. (Harvard), Associate Professor of Physiology, School of Medicine, *University of Rochester, Rochester, N. Y.*

Agersborg, H(elmer) P(areli von Wold) K(jerschow), B.S., M.S. (Washington), M. A. (Columbia), Ph.D. (Illinois), Consulting Biologist, *U. S. National Park Service, Box 378, Hilliard Road, Dumbarton, Va.*

ton), M.A. (Columbia), Ph.D. (Illinois), Senior Wild Life Technician, *National Park Service, Washington, D. C.*

Alexander, (Edward) Gordon, A.B. (Central), A.M., Ph.D. (Princeton), Associate Professor of Biology, *University of Colorado, Boulder, Colo.*

Allee, Warder Clyde, S.B. (Earlham), S.M., Ph.D. (Chicago), Professor of Zoölogy, *University of Chicago, Chicago, Ill.*

Allen, Bennet, Ph.B. (De Pauw), Ph.D. (Chicago), Professor of Zoölogy, University of California at Los Angeles, *909 Micheltorena St., Los Angeles, Calif.*

Allen, Edgar, A.M., Ph.D. (Brown), Professor of Anatomy, Yale University, *333 Cedar St., New Haven, Conn.*

Allen, Ezra, A.B., A.M. (Bucknell), Ph.D. (Pennsylvania), Associate Professor of Histology and Embryology, *New York Homeopathic Medical College and Flower Hospital, York Ave. and 64th St., New York, N. Y.*

Allen, William Ray, A.B., A.M., Ph.D. (Indiana), Professor of Zoölogy, University of Kentucky, *417 Clifton Ave., Lexington, Ky.*

Altenburg, Edgar, A.M., Ph.D. (Columbia), Assistant Professor of Biology, *Rice Institute, Houston, Texas.*

Anderson, Bertil G., A.B. (Augustana), M.S., Ph.D. (Iowa State), Instructor in Biology, *Biological Laboratory, Western Reserve University, Cleveland, Ohio.*

*Andrews, Ethan Allen, Ph.B. (Yale), Ph.D. (Johns Hopkins), Professor of Zoölogy, *Johns Hopkins University, Baltimore Md.*

Angulo y Gonzalez, Armando W., A.B., A.M., Ph.D. (Kansas), B.S., B.L. (Inst. Prov. de Oriente, Santiago de Cuba), Associate Member of *The Wistar Institute, Woodland Avenue and 36th Street, Philadelphia, Pa.*

ARFY, LESLIE BRAINDERD, Ph.D. (Harvard), Robert L. Rea Professor of Anatomy and Chairman of the Division of Anatomy, *Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill.*

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TOTALS

Life members,	5
Active members,	579
Associate members,	183
Grand total,	767

SIXTH MEETING OF THE 'INSTITUT INTERNATIONAL D'EMBRYOLOGIE'[1]

(Short communication of the Secretary-treasurer)

The International Institute of Embryology which promotes advancement of the comparative embryology of vertebrates by creating scientific and friendly connections between the principal investigators of that science and by founding a collection of rare embryological material and an international library, held it biennial meeting at Turin the thirty-first of August and the first of September of last year in the Anatomical Institute under the kind hospitality of Prof. G. Levi.

In spite of the fact that only seven members had lent an ear to the convocation of the board the meeting was very interesting from a scientific point of view and certainly may be considered profitable in respect to the purposes of the Institute.

In the private session the proposed changes in the statute were approved. The board resigned but was immediately reelected, as follows: J. Boeke (Utrecht), President; O. Grosser (Prague), Vice-president; D. De Lange (Utrecht), Secretary-treasurer.

The meeting made the proposal to bestow the honorary membership on six persons: F. Hochstetter (Vienna), Thomas H. Morgan (Columbia University, New York), A. Nicolas (Nancy), F. Graf von Spee (Kiel-Holtenau), J. T. Wilson (Cambridge, England), Edmund B. Wilson (Columbia University, New York) and to elect eight other persons as efficient members: A. J. P. Van Den Broek (Utrecht), Miss H. Fell (Cambridge, England), Carl Hartman (Baltimore), J. Holtfreter (Munich), N. K. Koltzoff (Moscow), Wilhelm von Möllendorff (Zurich), T. Terni (Padua), G. Wislocki (Harvard University, Cambridge, Massachusetts). These proposals were later approved by absent members.

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[1] The seat of the association is at the 'Hubrecht Laboratory,' Janskerkhof 2, Utrecht (Holland).

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Finally Doctor Levi expressed the wish that the Institute should propose one or two themes of investigation suitable for the scientific collaboration of the members of the Institute. He suggested, as a possible theme: "The influence of the constitution on embryological variations." Doctor Celestino da Costa had a predilection for the following theme: "A comparative investigation of the first stages of development in mammals and of the connections between the phenomena characterizing that period."

Two sessions were devoted to the demonstration of microscopical preparations and to that of films or diapositives. The program contained the following themes:

1. Young stages of the macaque by Doctor Streeter (Baltimore).
2. Young stages of Galago demidoffi by Doctor Gérard (Brussels).
3. Amniogenesis of the hedgehog by Doctor Celestino da Costa (Lisbon).
4. Development of the paraganglia in bats by Doctor Celestino da Costa (Lisbon).
5. Cinematographic demonstration of tissue cultures of spinal ganglia of chick and human embryos by Doctor Levi (Turin).
6. Microscopical preparations of fixed tissue cultures of spinal ganglia and of the rachidian bulb, colored by a slightly modified Cajal method by Doctor Levi (Turin).
7. The bursa ovarii of the hedgehog showing a well-developed connection between the rete ovarii and the epithelium of the ostium tubae or of the ovarial sac by Doctor De Lange (Utrecht).
8. Terminal reticulum of the peripheral sympathetic nerve system in fat cells, sebaceous glands, blood vessels and striated muscle fibers by Doctor Boeke (Utrecht).

The demonstrations were followed by interesting discussions on the interpretation of the observed phenomena. So this meeting may be considered a success notwithstanding the fact that only a few members of the society were present.

D. De Lange, *Secretary*
Utrecht, Holland

THE MENINGEAL RELATIONS OF THE HYPOPHYSIS CEREBRI

I. THE RELATIONS IN ADULT MAMMALS

GEORGE BERNAYS WISLOCKI
Department of Anatomy, Harvard Medical School, Boston

FOUR TEXT FIGURES AND THREE PLATES (TWELVE FIGURES)

The present report describes the morphological relations of the meninges of the hypophysis in a series of adult mammals. Since these relationships are determined by embryological factors, a subsequent report will present observations and discuss certain considerations regarding the development of the hypophysis and its meninges in a series of human embryos and fetuses.

Great uncertainty exists at the present time regarding the arrangement of the meninges about the hypophysis. Opinion varies widely in regard to the degree to which leptomeninges extend around the epithelial and neural portions of the gland. One extreme is represented by the view of Hughson ('24) based upon histological studies of dogs, as well as upon experiments, in which Prussian blue reagents were introduced into the cerebrospinal fluid. He arrived at the conclusion that the pituitary is surrounded by all three meningeal envelopes so that the subarachnoid space encloses the entire gland. This concept is incorporated for man in a diagram presented in a recent review by Bailey ('32). The latter writer also indicates that besides the layer of pia surrounding the epithelial lobe there is a continuation of this pial lining separating the pars intermedia and neural lobe.

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Atwell ('26), on the other hand, from study of the human hypophysis, leaves the matter open. In his well-known diagram he presents the pars tuberalis as enclosed in pia but indicates nothing definite about the envelopes surrounding the pars distalis or neural lobe. He represents no pia inside the sella.

Woerdeman ('18) states that the pia invests only the tissue of neural origin. Cowdry ('22) similarly accepts the pia as surrounding the neural lobe and the dura as enveloping the entire gland. Parker ('17) portrays the pia mater as investing only the pars neuralis and pars tuberalis in Trichosurus and Perameles, two marsupials, while the pars distalis is surrounded by dura mater only.

Stendell ('14) represents both pia mater and dura surrounding the neural lobe, whereas he believes the anterior lobe to be enveloped by capsule and periosteum lining the sella.

Brander ('32) indicates briefly that in the human he believes dura to exist between periosteum of the sphenoid and capsule of the pituitary. Koller ('22) describes dura lining the sella in various mammals, but touches upon the question of leptomeninges too briefly to reach any definite conclusions.

These citations illustrate the uncertainty of opinion in regard to these matters. The older views are presented in the paper by Hughson ('24).

Because of the unsettled state of this question, Schwartz ('36), working in this laboratory, undertook a reinvestigation of the meningeal relations of the hypophysis in dogs. By the combination of injection of carbon particles into the cerebrospinal fluid and of sections cut serially from the base of the skull with the pituitary completely in situ, he has brought forth convincing proof of the anatomical extent of the subarachnoid space in this region. He has demonstrated clearly that in the dog the subarachnoid space does not extend around the body of the hypophysis. His preparations show also that the extension of ink coincides exactly with the presumable limits of the subarachnoid as determined by the histological method of study of carefully prepared serial sections.

The present report is based upon a study of serial sections through the hypophyseal region of adult rats, rabbits, cats and rhesus monkeys.

METHODS AND MATERIAL

For purposes of study of the hypophyseal meninges in the adult, the base of the skull with the brain and hypophysis in situ was fixed and then decalcified in trichloracetic acid. Thus the hypophysis remained undisturbed, a necessary precaution in studying the topography of the membranes. To this mode of procedure is attributed the success of the present investigation. Hughson and others depended for examination of the gland upon specimens removed from the sella; this manipulation, even with the greatest of caution, produces unavoidable displacement and tearing of the meninges which preclude any clear demonstration of their relations.

The hypophyses subjected to study in this manner were from adult rats, rabbits, cats and rhesus monkeys. The appearance of these is presented in a series of photographs of the sections. Four text figures, moreover, give in a diagrammatic way the relationships of the meninges and hypophysis as derived from study of the material.

OBSERVATIONS

Adult monkey. In the rhesus monkey (figs. 1, 5, 6 and 7) the hypophysis lies in a deep sella. The dura is fused throughout the sella, on the one hand with the periosteum and on the other with the capsule of the body of the gland. There is consequently no subdural space inside the sella. Beneath the brain the pia-arachnoid and subdural space can be observed on either side of the infundibular stalk (figs. 1 and 6). The pia-arachnoid applies itself to the stalk in the form of a collar or cuff, the subarachnoid cistern encircling the neck of the stalk and not continuing around the body of the gland. The subdural space also extends as a collar encircling the pituitary stalk without any continuation down into the sella.

ABBREVIATIONS FOR ALL FIGURES

ar, arachnoid membrane
ba, basilar artery
di, diaphragm of sella
d, dura
hc, hypophyseal capsule
mb, mammillary body
mr, mammillary recess
oc, optic chiasma
p., periosteum
pd, pars distalis
pi, processus infundibuli (neural lobe)
pint, pars intermedia
pt, pars tuberalis
sas, subarachnoid space
sd, subdural space
v, vein
III V, third ventricle

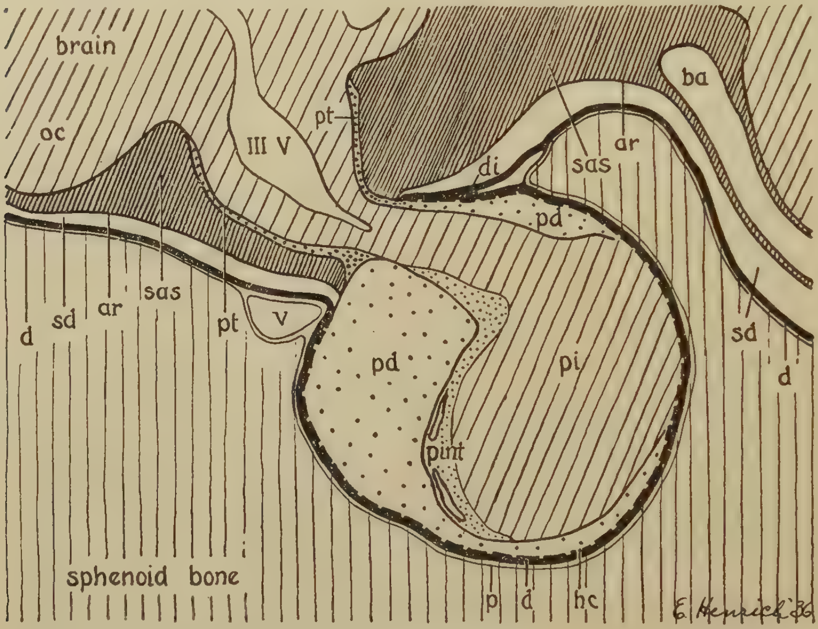

Fig. 1 Diagram of midsagittal section of hypophysis of rhesus monkey. For lettering consult list of abbreviations. Compare with photograph (fig. 6) of a similar midsaggital section of monkey's hypophysis. Notice that the leptomeninges do not surround the body of the pituitary gland. Instead, within the sella, periosteum (p), dura (d) and hypophyseal capsule (hc) have fused. Consequently the subdural space (sd) also does not exist there.

Figures 5 and 7 of sections to either side of the midsagittal plane show clearly that both subarachnoid and subdural spaces are limited to the suprasellar region in the vicinity of the stalk, and do not enclose the body of the gland located in the sella. Around the neck of the hypophyseal stalk (figs. 1 and 6) the dura fuses with the capsule of the body of the pituitary resulting in the absence of any subdural space between the body of the gland and the dura. The collar-like dural investiture at the transition between hypophyseal body and stalk creates the dural diaphragm of the sella (fig. 1, di).

Adult cat. In the cat (figs. 2, 8, 9 and 10) the arrangement of the meninges is essentially the same as in the monkey.

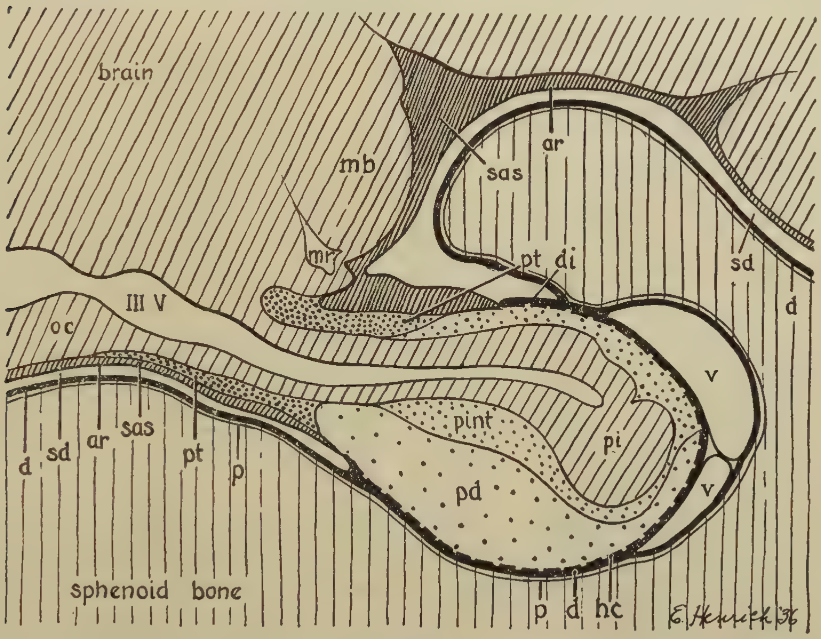

Fig. 2 Diagram of midsagittal section of hypophysis of cat. For lettering consult list of abbreviations. Compare with photograph (fig. 9) of a midsagittal section of cat's hypophysis. Observe that the arachnoid membrane (ar) and subarachnoid space (sas) surround the hypophyseal stalk, but do not penetrate the sellar recess. Periosteum (p), dura (d) and hypophyseal capsule (hc) are fused, so that the subdural space (sd) also does not extend around the hypophysis.

The sella is somewhat shallower and the anterior clinoid process is less well developed. The subarachnoid space encircles the hypophyseal stalk and the upper surface of the body of the gland, but does not extend any distance into the sella, excepting anteriorly where the pia-arachnoid is carried a short distance along the curving margin of the pars distalis.

Thus anteriorly the subarachnoid space dips in to form a small apron-like recess or cul-de-sac (figs. 2 and 9). The subdural space terminates on the anterior margin of the pars distalis a short distance below the line of attachment of the pia-arachnoid, while posteriorly it extends as a short cul-de-sac between the posterior clinoid process and the diaphragma sella covering the adjacent surface of the pituitary. The sellar diaphragm is a transverse sheet or fold of dura covering the superior aspect of the neural pole of the pituitary and intimately fused to the surface of the gland (fig. 2, di), the latter circumstance thus precluding any possible extension of the subdural space posteriorly or laterally beneath the pituitary. Periosteum, dura and capsule of the gland are fused with one another within the sellar recess. The photographs (figs. 8, 9 and 10) show the connective tissue in this zone of fusion as separated into individual bundles of frayed fibers. This is an artefact due to shrinkage during dehydration of the specimen. In another series from the cat, not illustrated here, there has been no shrinkage, so that capsule, dura and periosteum consist of an uninterrupted lamina.

Adult rabbit. In the rabbit (figs. 3, 11 and 12), as in the previous animals, the pituitary stalk which is rather long and slender is encircled by a closed pia-arachnoidal cistern which does not exhibit anatomical continuity with any intrasellar spaces. Indeed the subarachnoid space ceases on the stalk proper (fig. 3) without any extension whatsoever onto the body of the pituitary as in the cat (fig. 2). The subdural space also extends around the pituitary stalk, but is prevented from penetrating the sellar fossa by the fact that the sellar diaphragm is fused intimately with the superior surface of the body of the pituitary gland. The periosteum and dura are fused with the capsule of the pituitary throughout the entire sellar recess. Separation between dura and pituitary occurs readily here and there due to shrinkage, giving in some places the false impression of the actual existence of isolated subdural spaces. These appearances are regarded as artefacts, this opinion being based upon study of a number of series of rabbits' pituitaries from which it becomes amply clear that neither isolated subdural recesses nor intrasellar extensions from the normal suprasellar subdural space actually exist.

Adult rat. In the rat, although at first glance the topography of the hypophyseal region seems very different (figs. 4, 13 and 14), the fundamental relationships of meninges to hypophysis are very similar to those described in the previous animals. The rat possesses no more than a shallow depression in the sphenoid bone in which the pituitary rests. Lacking a true sellar fossa, the pituitary appears to have

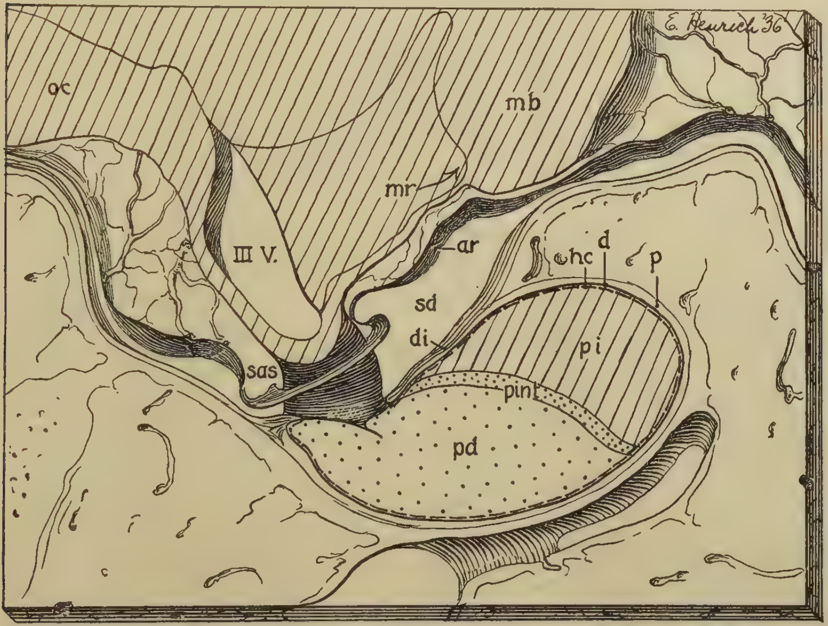

Fig. 3 Semidiagrammatic drawing of a thick (300 μ) parasagittal section of hypophysis of rabbit. For lettering consult list of abbreviations. Compare with photographs (figs. 11 and 12) of sagittal and parasagittal sections of rabbit's hypophysis. Note especially the collar-like arrangement of the arachnoid membrane (ar) around the hypophyseal stalk, in consequence of which the subarachnoid space is closed off from communication with the intrasellar structures. Notice also that the subdural space (sd) does not penetrate the sella turcica, but that, instead, periosteum (p), dura (d) and hypophyseal capsule (hc) are fused.

been drawn or pushed caudally so that it lies in the interpeduncular fossa beneath and behind the mammillary body. It is apparent from examination of serial sections that the pituitary is attached firmly to the shallow depression of bone in which it lies by a fusion of its capsule with the underlying dura and periosteum (fig. 4). There is no possibility that either subdural or subarachnoid space penetrates this bed of attachment of pituitary to the sphenoid bone. It is also clear upon study of the sections that a delicate lamina of fused dura and hypophyseal stroma surrounds the entire gland including the infundibular process. The pia-arachnoid in the

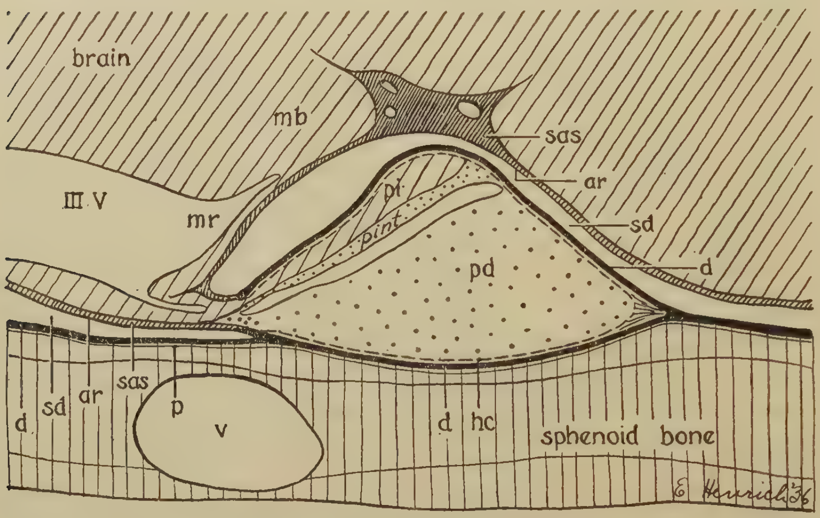

Fig. 4 Diagram of midsagittal section of hypophysis of rat. For lettering consult list of abbreviations. Compare with photographs (figs. 13 and 14) of sagittal sections through rat's hypophysis. Note particularly in the rat that the pituitary lies upon the flattened sphenoid bone to which it is attached by the fusion of the hypophyseal capsule (hc), dura (d) and periosteum (p). Notice also that the pituitary, excepting stalk, is entirely enclosed in dura (d). Lacking a sella turcica, the hypophysis of the rat appears to have become lodged or drawn into the interpeduncular fossa behind the mammillary bodies (mb). In spite of its different topography, the arrangement of the hypophyseal meninges is essentially the same as in the preceding animals, in that neither subarachnoid (sas) nor subdural space (sd) intervenes between the capsule of the hypophysis (hc) and the dura (d).

photographs (figs. 13 and 14) is collapsed, following the contours of the brain as a dark wavy line. The delicate pia-arachnoid encloses the stalk of the pituitary as far as the junction of stalk and body where the dural capsule begins. In the photographs the subdural space is conspicuous between the pia-arachnoid, outlining the contours of the brain, and the dura which covers the sphenoid bone and encapsulates the pituitary gland.

Human fetus. Two photographs (figs. 15 and 16) of a human pituitary from a fetus of 16 cm. sitting height are introduced here for comparison with the previous series of mammals. It has not been possible to obtain a comparable adult human pituitary in situ, but the meninges in the present, well-advanced fetal stage show the essential similarity of the topography of the meninges in man to that of the other mammalian forms. The pictures show that at this stage the body of the pituitary rests in a vascularized cushion of dural connective tissue occupying the sella turcica. This connective tissue unites the surface of the hypophysis with the periosteum of the sella without the intervention of either subarachnoid or subdural space. The subarachnoid space is clearly visible, however, encircling the hypophyseal stalk beneath the brain. It is in no way connected with the contents of the sella. The findings in this late fetal stage in the human coincide well with the topography of the adult animals which have been investigated.

DISCUSSION

We conclude from our observations that in the mammals investigated neither subarachnoid space nor subdural space actually surrounds the body of the pituitary or extends far into the sella turcica when the latter is present. The subarachnoid space encircles the pituitary stalk and is limited to that region. The dura clothes the entire wall of the sella fusing on the one hand with the surface of the body of the pituitary and with periosteum on the other. Consequently there is no subdural space represented within the sella.

These findings are diametrically opposed to the results of Hughson ('24). However, Hughson based his conclusions mainly upon physiological rather than morphological evidence, namely, the demonstration that in dogs the Prussian blue reagents introduced into the subarachnoid space find their way into the tissue surrounding the body of the hypophysis in case the cerebrospinal fluid pressure is experimentally altered.

Recent observations, however, by Schwartz ('36) on dogs, referred to in the introduction, are totally at variance with Hughson's findings. Schwartz introduced a suspension of carbon (Higgins waterproof India ink) into the subarachnoid space of living dogs. At autopsy he was able to trace clearly the carbon to the subarachnoid cistern surrounding the hypophyseal stalk, but not into the tissues within the sella surrounding the body of the hypophysis. In his sections the anatomical limits of the subarachnoid space are convincingly outlined by the deposited carbon. Consequently, he reaches the conclusion that in the dog the subarachnoid space does not extend around the body of the pituitary. From Schwartz's morphological, as well as physiological, evidence it appears unlikely that Hughson's experience with Prussian blue should be interpreted as evidence of the extension of the subarachnoid around the body of the hypophysis. The most likely interpretation of Hughson's results is that under abnormal cerebrospinal fluid pressures fluid may diffuse out into the dural tissues encapsulating the pituitary. There is much evidence to support such an explanation. The arrangement of meninges around the pituitary is almost identical with that occurring around the spinal nerve roots. Elman ('23) has shown that around these nerve roots, where pia-arachnoid and dura end, the Prussian blue reagents leave the confines of the subarachnoid space to diffuse into the dura escaping thence into dural veins which are plentiful in this vicinity. In the pituitary region of the dog, as Schwartz has pointed out, the relations of pia-arachnoid, dura and dural veins are analogous to the spinal roots. From the work of

Schwartz, as well as from the present observations, it is concluded that the finding of Prussian blue within the sella by Hughson demonstrates not an antomical continuity of the subarachnoid space around the body of the pituitary, but merely the possibility, under conditions of heightened escape of cerebrospinal fluid, of the diffusion of cerebrospinal fluid into the adjacent periaxial dural tissue.

Schwartz's findings, after introducing carbon into the subarachnoid, show, moreover, that the distribution of foreign particulate matter coincides exactly with the anatomical limits of the subarachnoid cistern as traced without resort to injection. Thus the morphological picture of the extent of the subarachnoid space can be relied upon as an accurate gauge of the actual anatomical confines of the space. As a result of Schwartz's experiment, we regard the morphological method here employed to trace the extent of the subarachnoid space in various mammals as valid. Indeed the topography of the membranes around the hypophysis of the cat is practically identical in every detail with the arrangements observed in the dog.

The interpretation of the extent of dura and pia-arachnoid adopted here is, moreover, strengthened in some particulars by observations which were made on serially sectioned hypophyses of a cat, rabbit and monkey which were stained intravitally with trypan blue. It is well known that the dura stains quickly and intensely with trypan blue, whereas the pia-arachnoid stains slowly and feebly. In all three of these animals the results in the sellar region after vital staining are identical. The dura can be traced by virtue of its deep blue staining, forming the diaphragm of the sella and thence extending into the cavity of the sella. It has been observed that the lamina of tissue which encapsulates the pituitary body and binds the pituitary to the bony wall of the sella, which we interpret as dura, is deeply colored with the vital dye, partly by virtue of a diffuse blue infiltration of the dural tissue, and to a lesser extent by the presence of vitally stained macrophages. This suggests that the pia-arachnoid is not involved in the formation of the intrasellar capsular formation. Nevertheless, how far the stroma of the leptomeninges may penetrate as a consolidated membrane or as diffuse connective tissue, unassociated with subarachnoid spaces, into or around the anterior hypophysis, or between the neural lobe and the buccal derivatives of the pituitary, is difficult to predicate from the present material. Some significant information about this question can be obtained, however, from embryological study of the hypophysis, the results of which will be presented in a subsequent paper of this series.

It appears, with individual variations, that the topography and extent of the subarachnoid space are fundamentally similar in all of the mammals which have been drawn into the present investigation. As described in previous papers, however, the hypophysis of one group of mammals, namely, the whales, is constituted very differently from the animals in the present series. Wislocki ('29) and Wislocki and Geiling ('36) have shown that the whale's pituitary consists of a neural and of a buccal portion which are anatomically separated excepting a slender pars tuberalis which clasps the infundibular stalk. In whales the anterior lobe of the pituitary lies firmly embedded in dura without the interposition of either leptomeninges or subdural space, in this respect resembling the present observations on other mammals. The anatomically separated infundibular process of whales, on the contrary, is enclosed by a capsule which appears to split on the infundibular stalk and to become continuous with the arachnoid and pia respectively. Thus the capsule of the neural lobe is to all intents and purposes a membranous continuation of the leptomeninges. Furthermore, there exists in whales a well-defined subdural space and dural recess in which the independent neural lobe is pocketed. Consequently the neural lobe is effectively separated by dura and subdural space from contact with the anterior lobe. Thus, due to the unusual relations of the buccal and neural portions of the pituitary in whales, the pattern of the meninges departs in certain regards from the arrangements encountered in other mammalian groups.

An attempt will be made in the succeeding paper on the embryology of the human pituitary region to trace the development of certain of the meningeal relations observed in the adult stage.

SUMMARY AND CONCLUSIONS

1. A method of studying the topography of the adult pituitary region is presented. This consists, after decalcification, in serially sectioning the base of the brain and skull with the pituitary completely in situ. The reasons are given for considering the observations derived from pituitaries studied by this method as more trustworthy than previous observations.
2. The conclusion is reached that in a variety of mammals (rat, rabbit, cat, dog, monkey and man) the subarachnoid space encircles the infundibular stalk, but does not surround the body of the hypophysis. The subarachnoid space penetrates the sella turcica for only a short distance, anteriorly, in dog and cat, but not at all in rabbit, monkey and man.
3. The subdural space, similarly, does not surround the body of the pituitary. The capsule of the pituitary gland, the intrasellar dura and the adjacent periosteum constitute a continuous fused lamina, precluding extension into the sella of either the subarachnoid or subdural space. Shrinkage during dehydration of specimens often causes clefts to arise in the dural stroma between the pituitary and the surrounding bone of the sella turcica. These are artefacts, as proved by favorable specimens in which shrinkage is minimal.

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PLATES

PLATE 1

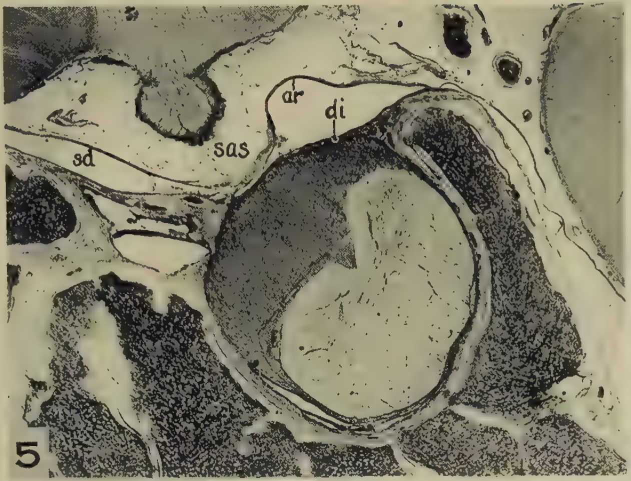
EXPLANATION OF FIGURES

5 Parasagittal section of hypophysis of rhesus monkey. Note the arachnoid (ar) and subarachnoid space (sas) extending over the top of the hypophysis. × 12.

6 Midsagittal section of hypophysis of rhesus monkey. For interpretation compare with figure 1. Note the collar-like topography of arachnoid (ar) and subarachnoid space (sas) about the hypophyseal stalk. × 12.

8 Parasagittal section of hypophysis of cat. Note broad attachment of the pia-arachnoid in the stalk region to the surface of the anterior lobe. × 12.

9 Midsagittal section of hypophysis of cat. For interpretation compare with figure 2 of a similar section. × 12.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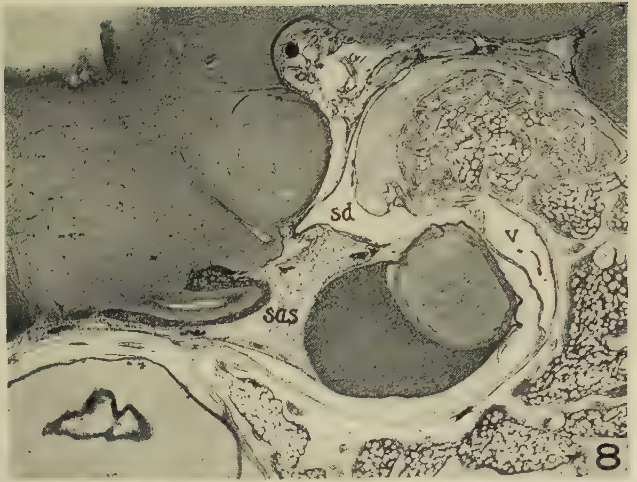
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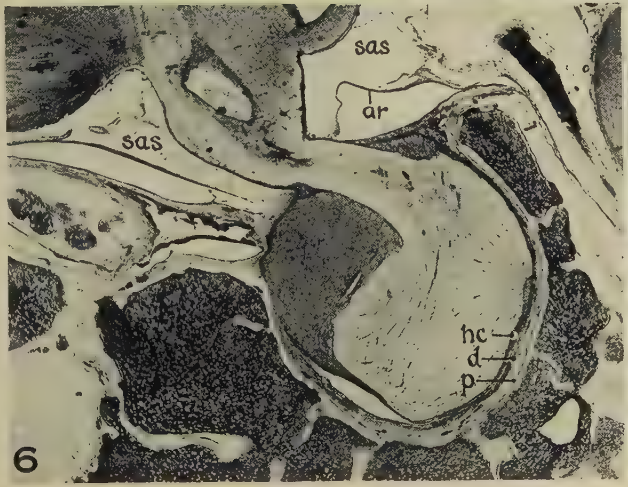
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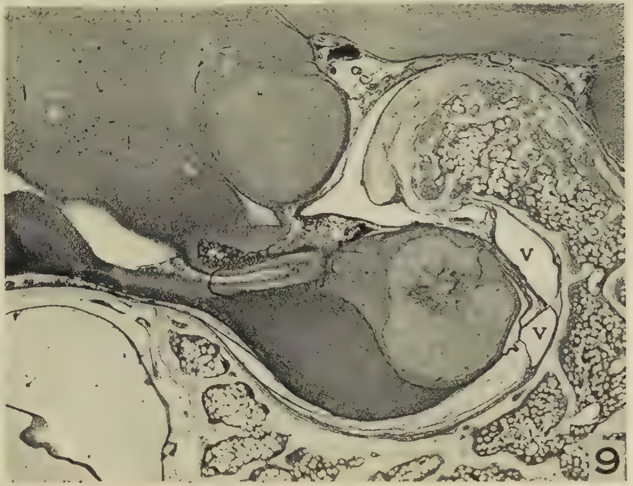
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PLATE 2

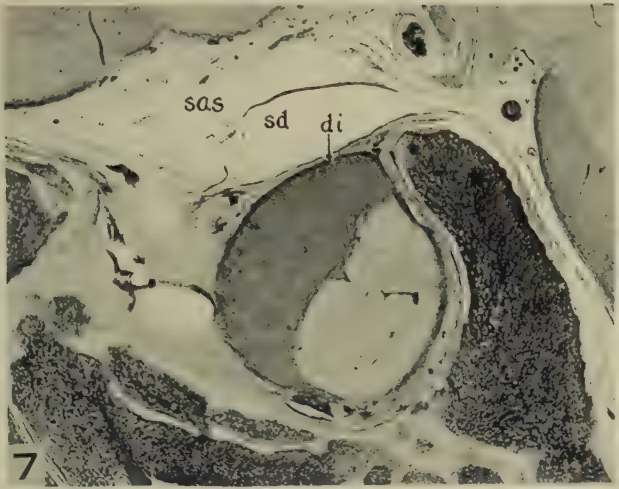
EXPLANATION OF FIGURES

7 Parasagittal section of hypophysis of rhesus monkey. In this and the two previous figures observe the fusion of hypophyseal capsule, dura and sellar periosteum. × 12.

10 Parasagittal section of hypophysis of cat. Compare with figure 2. × 12.

11 Parasagittal section of hypophysis of rabbit. Note wavy arachnoid membrane (ar) with subdural space (sd) intervening between it and the surface of the pituitary gland. × 12.

12 Midsagittal section of hypophysis of rabbit. Compare this and previous section (fig. 11) of rabbit with the semidiagrammatic drawing presented in figure 3. × 12.



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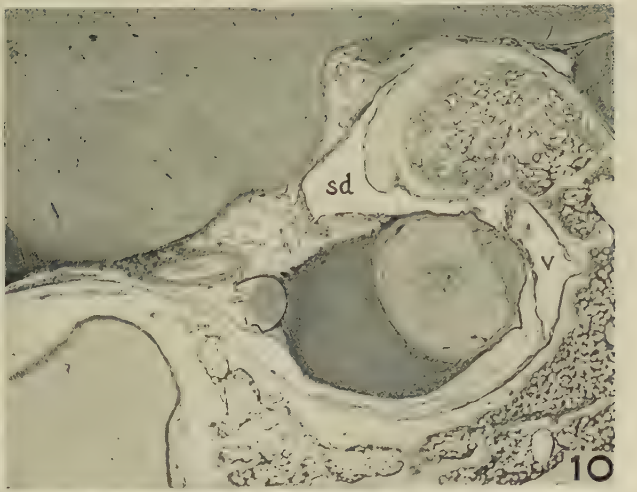

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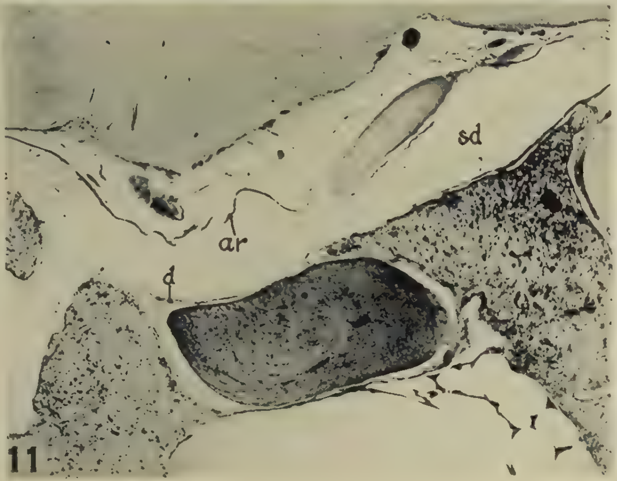

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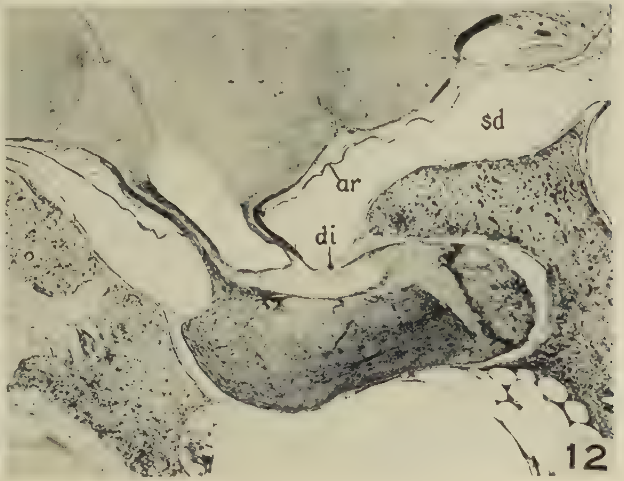

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PLATE 3

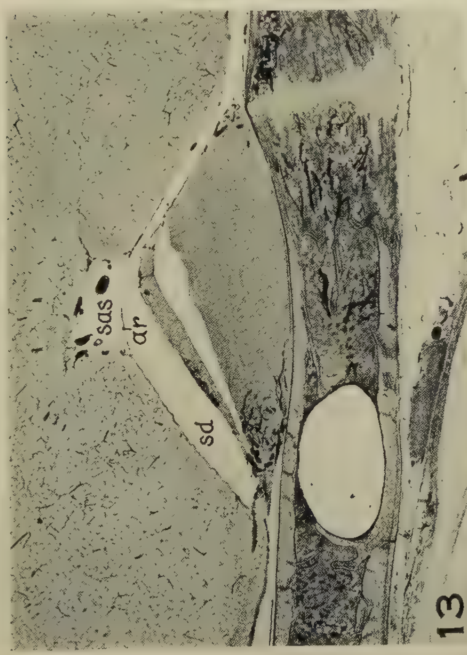
EXPLANATION OF FIGURES

13 Sagittal section, close to the midline, of hypophysis of rat. For interpretation compare with figure 4. × 18.

14 Parasagittal section of hypophysis of rat. In this and preceding section (fig. 13) of rat notice fusion of periosteum, dura and hypophyseal capsule beneath the pituitary gland. × 18.

15 Sagittal section, near the midline, of hypophysis of human fetus (16 cm. sitting height). Notice that the body of the pituitary within the sella is firmly embedded in dural connective tissue. The pia-arachnoid (ar) and subarachnoid space (sas) encircle the pituitary stalk and are sharply delimited from the dura which occupies the sella and envelopes the body of the hypophysis. × 18.

16 Parasagittal section of the same fetal human hypophysis, showing clearly the sharp separation of the subarachnoid space (sas) from the contents of the sella turcica. × 18.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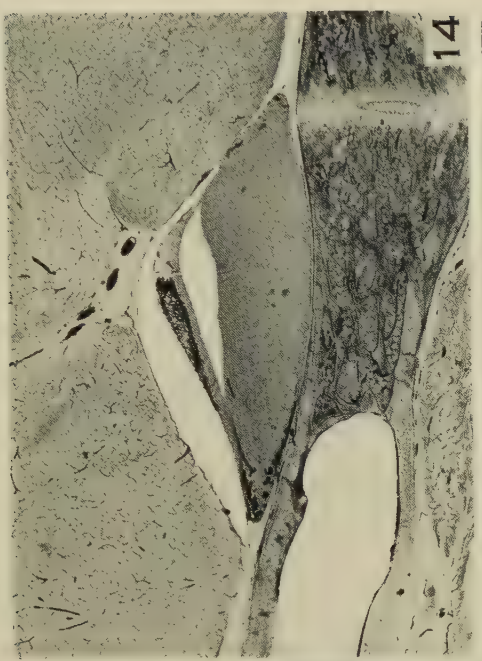
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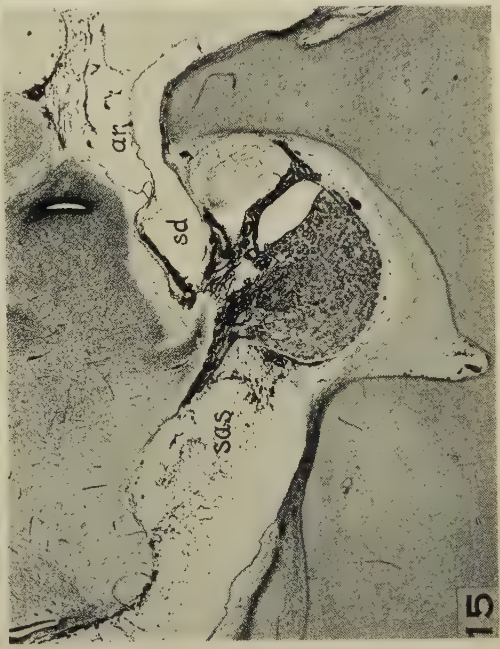
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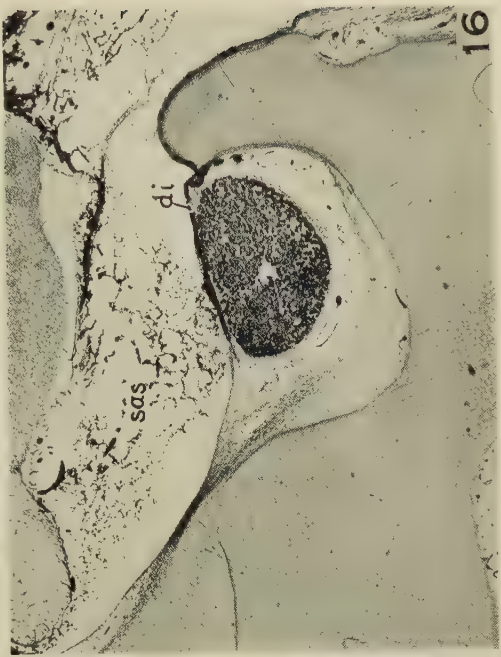
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ON THE NATURE OF THE SO-CALLED DROPLETS FOUND BETWEEN THE ROD OUTER SEGMENTS OF VERTEBRATE EYES

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ONE PLATE (SIX FIGURES)

INTRODUCTION

When the vertebrate retina is fixed with modified Held's solution (v. Kolmer, '09) and stained subsequently with iron hematoxylin, there are seen between the rod outer segments and the epithelial pigment layer, deeply stained spherical or ovoid granules or 'droplets' which are usually abundant in dark-adapted eyes and typically more sparse in light-adapted eyes. Kolmer (op. cit.) who first described these 'droplets' regarded them as secretory products of the pigment epithelium and was led to the conclusion that they are, in some way, concerned with the visual purple function of the rods.

In an experimental study of the gecko retina (Detwiler, '23 a) Kolmer's observations were substantiated in that these droplets were more abundant in dark-adapted eyes than in light-adapted eyes. It became apparent, however, from this study, that these droplets do not have their origin in the pigment epithelial cells, but are products of the rod outer segments. In light-adapted eyes, when the outer segments were found to be backed with deeply staining striated material, the droplets were sparse. In dark-adapted eyes, when the droplets were abundant, the deeply stained striated material in the outer segment was sparse or lacking.

In a later communication, Kolmer ('25) was able to change his original idea regarding the origin of these droplets, and he supported Detwiler's view that they probably do not arise from the pigment epithelial cell. Final and conclusive proof for this contention was furnished by Johnson ('35) who grafted heterotopically the inner layer of the embryonic optic cup of Amblystoma, and obtained a certain number of cases with differentiated retinas which lacked a pigment epithelium. In such eyes droplets were found associated with the differentiated rod outer segments.

In an experimental study of the developing rat retina (Detwiler, '32), droplets in the fixed retinae could not be detected until the period when visual purple was first seen in the fresh isolated dark-adapted eye. This correlation was first observed at about 12 to 13 days after birth, and suggested still more strongly that the droplets are in some way associated with visual purple physiology. In the pure cone retina of lizards and tortoises, the droplets are entirely absent (Detwiler, '23 b; Kolmer, '25), although the latter author claimed to have observed them upon the foveal cones of the chimpanzee and man. The possible significance of this observation has already been discussed (v. Detwiler, '23 a). Kolmer also suggested the lipoidal nature of these droplets.

Our present interest in a further study of these droplets was in part incited by the statement of Walls ('34) who says "this mutual exclusiveness of the 'droplets' and 'striations' is interesting, but a relation of these phenomena to each other or of either to rhodopsin is unlikely. I consider 'Kolmer's droplets' to be artefacts and have yet to see them in sections of any type of retina embedded in pyroxylin and stained as Detwiler recommends."

Since the technique used by Kolmer, Detwiler and Johnson is similar to that of Weigert (1884) for staining myelin in celloidin or paraffin section, it seemed desirable to study the retina with the view of investigating the possible lipoid nature of the droplets as suggested by Kolmer ('25), and the following experiments were therefore undertaken.

MATERIALS AND METHODS

Adult male frogs (Rana pipiens) were placed in a dark room from 3 to 6 hours. The eyes were extracted and fixed under faint red light (Wratten filter, series 2). One eye was placed in modified Held's solution (Detwiler, '23 a) for paraffin or celloidin embedding; the other in neutral formalin for use in the gelatin-freezing technique (Zwemer, '33).

The gelatin-embedded sections were stained with one of a number of fat stains, counterstained with Harris hematoxylin for the nuclei and in some cases with malachite green for cytoplasmic detail and mounted in glychrogel (Wotton and Zwemer, '35).

Frozen sections without gelatin embedding were also made. Although the droplets could be observed, the breakage and distortion of the rod outer segments were too great to permit of accurate study so this technique was abandoned.

Fat staining in toto for 24 to 48 hours in Sudan III, after formalin fixation and before gelatin embedding was of value in reducing the amount of handling necessary for the cut gelatin or frozen sections. The paraffin and celloidin sections were stained with iron hematoxylin (compare Detwiler, Weigert) and also with Sudan III or IV (Ciaccio, '05).

OBSERVATIONS

Between the outer segments of the rods small 'droplets' of various sizes can be readily seen when using magnifications of × 450 or more. These droplets are sometimes aggregated in masses or clumps, and in the frog retina they are typically more abundant between the proximal portions of the outer segments, although in many cases they can be seen throughout the entire region between the outer rod segments (plate 1). When the sections are treated with iron hematoxylin, the 'droplets' show varying intensities of stain, ranging from very light gray through a blue color to almost black such as is found in the staining of myelin.

With Sudan III the droplets take a reddish-yellow stain in eyes that have been sectioned by freezing or by the gelatin-freezing methods following formalin fixation (fig. 1). They stain similarly with Sudan III or IV in paraffin or celloidin sections (figs. 3 and 5) provided the tissues have been adequately chromated before embedding. The failure to find droplets in certain instances in both paraffin and celloidin sections following fixation with modified Held's solution is regarded as due to faulty technique which does not provide for the proper chromation of lipoids.

The method for staining the droplets with iron hematoxylin (Detwiler, '23 a) has been successfully applied to celloidin sections (fig. 6) and has been repeatedly used with paraffin sections of adult frog eyes (fig. 4) and in 'rosettes' of grafted larval eyes of Amblystoma lacking pigment epithelium (Johnson, '35). The droplets in rosettes also stain with Sudan III (fig. 2).

DISCUSSION

The technique employed by Kolmer and Detwiler for demonstrating the so-called 'droplets' between the outer segments of the rods in vertebrate eyes, especially following dark-adaptation is similar to that introduced by Weigert for myelin, since in both cases the preservation of the lipoids from the effect of organic solvents has been by chromation. The chromic acid released from the bichromate salt in an acid solution probably acts on the double bonds of unsaturated fatty acids to form compounds with reduced solubility in lipoid solvents, which at one stage of their oxidation have the property of forming a dark blue lake with hematoxylin.

The Weigert technique has been shown by Wlassak (1898) to be especially good for staining cephalin (protagon), with the given conditions of fixation—other lipoids being poorly stained or entirely refractory. Similarly Ciaccio ('05) has found that lipoids protected from the paraffin embedding procedure by chromation can be stained with specific fat stains such as Sudan III. With this stain many of the fats are red, but cephalin is reddish-yellow. It is not surprising that the 'droplets' in question, being associated with cells of neural origin, act similarly to cephalin, staining blue-black with iron hematoxylin and reddish-yellow with Sudan III.

Our observations which show that these 'droplets' can be found in frozen, gelatin, paraffin and celloidin sections, do not support the contention of Walls that they are artefacts produced in embedding.

From careful microscopic study one gets the impression that in some instances the droplets appear to be partly within and partly outside the rod outer segments. If it could be demonstrated that they actually migrate per se, the phenomenon would be comparable to the results of recent studies on the ability of intestinal, liver (Zwemer and Wotton, unpublished), and adrenal (Singer and Zwemer, '34) cells to take in or discharge fats in visible droplet form. Proof, however, of an actual discharge of these droplets from the rod is still wanting.

The fact that the materials in the rod outer segments and the droplets are rich in lipoid strengthens the view expressed by Kolmer and Detwiler that they are in some way associated with visual purple (rhodopsin). The latter is closely associated with visual yellow (retinene) and vitamin A, which are present in inverse proportions to visual purple in its changes on exposure to light and subsequent recovery in darkness (Wald, '35). Both retinene and vitamin A are typical lipoids soluble in organic solvents. The reversible changes between visual purple and retinene may well take place in this lipoidal portion of the rod cells, since both the droplets (Johnson) and rhodopsin (Tansley, '33) have been shown to be associated with the rod outer segments and not with the pigment epithelium. The relation of lipoid-soluble vitamin A to night vision might be given a histologic basis by studying the rod lipoids in dark-adapted, vitamin A deficient animals suffering from night blindness.[1]

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[1] A study of the 'droplets' in vitamin A deficient animals is being carried out by Johnson (personal communication).

SUMMARY

1. The 'droplets' found between the rod outer segments in vertebrate eyes, especially following dark-adaptation, described previously by Kolmer ('09, '25) and Detwiler ('23 a, '32) have been shown to be lipoid droplets staining similarly to cephalin.

2. With paraffin and celloidin embedding it is necessary to subject the eyes to careful chromation before embedding in order to protect the lipoid material, both inside and outside the rod outer segment, from the solvent action of the organic solvents (alcohol, xylol, ether) usually employed, and yet preserve the ability to stain with iron hematoxylin.

3. The 'droplets' have been demonstrated in eyes fixed in neutral formalin, stained with Sudan III or IV, sectioned with the gelatin-freezing technique and mounted in glychrogel, according to the method of Zwemer ('33).

4. Our studies do not, so far, support the contention of Walls ('34) that the observed droplets are artefacts due to paraffin embedding. We still believe from our studies that they are in some way associated with rod physiology and the possible relation of the droplets to visual purple is briefly discussed.

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PLATE 1

EXPLANATION OF FIGURES

1 Dark-adapted frog eye. Gelatin-frozen section; Sudan III bulk stain; formalin fixation. × 1200.

2 Transplanted Amblystoma eye 'rosette.' Paraffin section; Sudan III; Harris' hematoxylin; modified Held's fixation. × 1200.

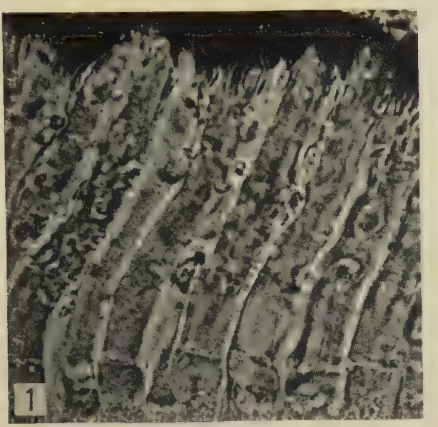
3 Dark-adapted frog eye. Paraffin section; Sudan IV; Harris' hematoxylin; malachite green; modified Held's fixation. × 1200.

4 Dark-adapted frog eye. Paraffin section; iron hematoxylin; erythrosin B; modified Held's fixation. × 1200.

5 Dark-adapted frog eye. Celloidin section; Sudan III; Harris' hematoxylin; modified Held's fixation. × 1200.

6 Dark-adapted frog eye. Celloidin section; iron hematoxylin; erythrosin B; modified Held's fixation. × 1200.

(Actual magnification × 1800; when reduced × 1200.)



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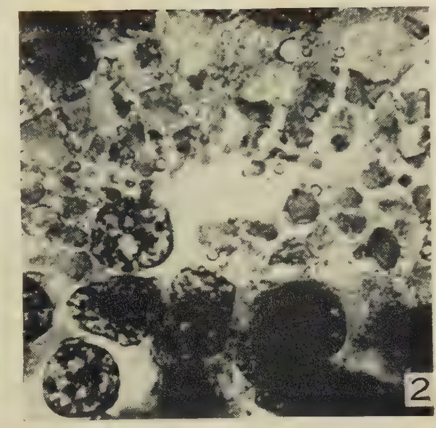

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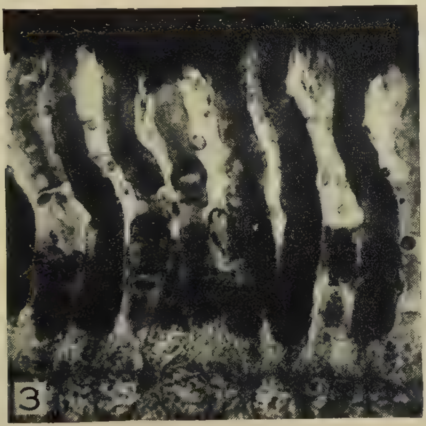

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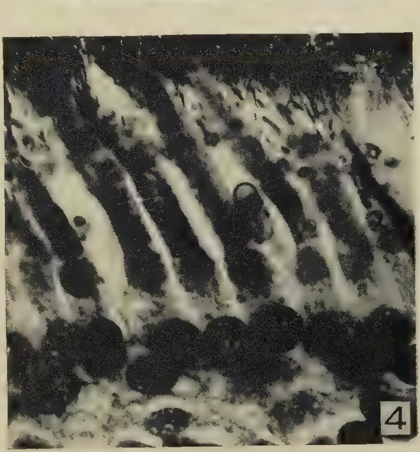

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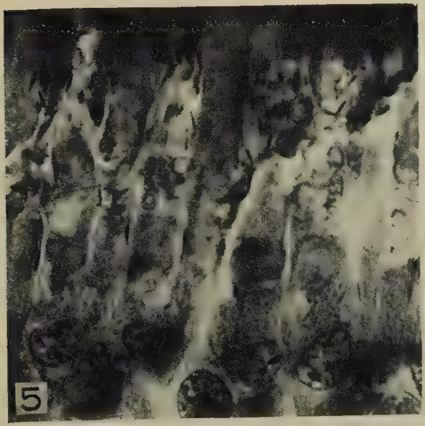

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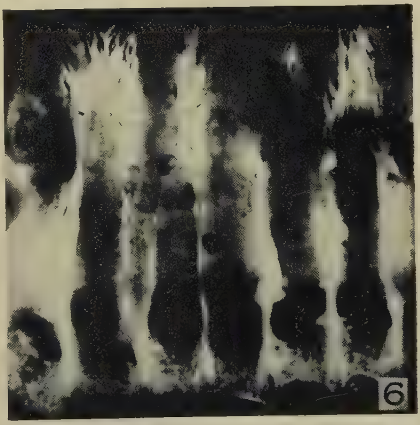

6

THE VAGINAL SMEAR PICTURE, SEXUAL RECEPTIVITY AND THE TIME OF OVULATION IN THE GUINEA PIG[1]

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ONE FIGURE

INTRODUCTION

From the standpoint of their value as points of reference for morphological, physiological and embryological investigations, it is probable that in the guinea pig no events in the reproductive cycle are more important than the vaginal changes, oestrus in the sense of heat or sexual receptivity and ovulation. Of the three, the vaginal condition has been given a place of primary importance, partly because it has been assumed to be the most readily determined, partly because the time relationships of a given vaginal smear picture to the time of heat and ovulation have been assumed to be definite (Stockard and Papanicolaou, '17 and '19; and Papanicolaou, '33), and partly because the appearance of a given vaginal condition in the absence of a mating test has come to be so generally regarded as presumptive evidence of heat.

Until circumstances enabled us to investigate the structural and behavioral changes at heat by means of continuous, day and night observations, these conclusions were regarded as more or less axiomatic. The first intimation that they might in any way be questioned was given by an early observation that following frequent vaginal stimulation, the time of heat occurred later with respect to the vaginal condition than

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[1] This investigation was supported by a grant from the Committee for Research in Problems of Sex, National Research Council.

normally (Young, Myers, Dempsey and Jennison, '34). Even then it was believed that the relationship between the vaginal smear picture and heat in normal animals was essentially as described. Within the last 2 years, however, it has been learned that important variations do occur (Young, Dempsey and Myers, '35) and it now seems that quite a different orientation of thought with respect to these relationships is called for.

Briefly, what has been found for the guinea pig is as follows: 1) The time of heat or sexual receptivity rather than the vaginal condition is the most readily determined of the oestrous phenomena (Young, Dempsey and Myers, '35); 2) the most definite relationship is that between the time of heat determined by behavioral signs and ovulation (Myers, Young and Dempsey, '36); 3) no one vaginal smear picture is associated with the occurrence of heat, therefore this method is not satisfactory for determining the time of heat; 4) the vaginal condition cannot be relied upon to indicate the exact time of ovulation, and 5) with the exception of one hitherto unsuspected relationship, the chief values of the vaginal smear technique for this species would appear to be in determining the existence of a reproductive cycle and possibly as a continued means of assay of oestrogenic preparations. Beyond this too much dependence should not be placed upon it.

The relationship referred to above is between the length of heat in many animals and the vaginal condition when heat begins. When animals come into heat late in stage I of Stockard and Papanicolaou (our stage Ib, late), its length often is significantly shorter than it is when they come into heat early in stage I (our stage Ia). The possible value of this relationship lies in the clue it may provide in a study of the factors controlling the length of heat.

Upon first thought the conclusions given above would seem to constitute a rather serious indictment of a technique that has been employed so generally and to such useful purpose. What is intended, however, is not the suggestion that the technique be given up entirely, but that as far as the guinea pig is concerned its limitations and further possibilities be better understood.

MATERIALS AND METHODS

The data presented in the following sections have accumulated during three experimental periods and from three colonies of animals. The experimental period for each colony was that time when the animals were being observed continuously, day and night, February 15 to May 15, 1933 for the first colony, October 15 to December 15, 1933 for the second colony, and October 10, 1935 to March 31, 1936 for the third colony. In no group of animals did the results differ from those obtained in other groups. They are, therefore, presented and discussed together.

The entire procedure was part of a program which comprehended several morphological and behavioral observations of numbers of animals at half-hourly intervals. For this reason it was impossible to do more than withdraw the vaginal fluid with a clean medicine dropper, spread it on a slide and allow it to dry. Later the smears were stained with Delafield's hematoxylin and eosin and studied. For the purpose in mind, namely, association of the vaginal condition with sexual receptivity and ovulation, it is probable that little would have been gained by better fixation.

The largest group of smears consists of 962 sets of two smears each, one made within a half hour after the beginning of heat, the other within a half hour after the end of heat. In this group the beginning of heat could not have been influenced by vaginal stimulation. Consequently, the normal relationships have been preserved as nearly as possible in a study of this kind. In nearly every case the length of oestrus is known and in 548 cases the length of the preceding dioestrum also is known.

The second group is composed of series of smears made from thirty-three animals. The first smears were made when the vaginal membrane opened and others at half-hourly or hourly intervals until the stage III condition had appeared, or in a few instances, only until several hours after heat regardless of the vaginal condition at that time. When heat occurred its duration is known for each animal. Had this method of making smears had no effect on the vagina, an exact association of the vaginal changes and heat would have been possible. As it is, allowance must be made for this factor and the relationships found between the vaginal condition and heat probably are not identical with those occurring in normal undisturbed animals.

To be added to this group for purposes of summarization are eleven animals whose vaginae were stimulated by insertion of a small glass rod at half-hourly intervals from the time of opening of the vaginal membrane until heat. A smear was then made which indicated the vaginal condition at that time.

A third group is composed of four sets of two smears each made from four animals injected intravenously with the pregnancy urine concentrate antophysin on the thirteenth or fourteenth day of the cycle. This was done for the purpose of inducing heat and ovulation prematurely and associating these oestrous phenomena produced experimentally with the vaginal condition.

The fourth group is composed of eighty-one single smears made when the animals were killed at the end of oestrus or within 3 hours after its end. In thirty-three of these subsequent microscopic examination of the ovaries revealed that rupture of at least one follicle had occurred. These smears, therefore, made possible an association of the vaginal condition with ovulation.

A last group was obtained fortuitously. Occasionally guinea pigs display a 'split oestrus' (Young, Dempsey and Myers, '35). In three instances of this smears were obtained at the beginning and end of each part of heat. In three others the smears are complete at the extremes, but no smear was made when the second or third part of heat began.

It is regretted greatly that a departure from the terminology used by Stockard and Papanicolaou and later by

Papanicolaou has seemed desirable. That some modification was called for is indicated by Papanicolaou's subdivision of stage I into Ia composed of many cornified cells mixed with superficial cells, and stage Ib composed of a prevalence of cornified cells. We have found, however, that another division based upon somewhat more definite end-points is possible. Our stage Ia is that part of the older stage I when superficial cells only are present, cornified cells not yet having appeared. As soon as epithelial sheets composed of two or three cornified cells could be found, the smears were classified as stage Ib, divided into Ib, early, when less than about 25% of the cells were cornified; Ib, middle, when approximately 25% to 75% of the cells were cornified; and Ib, late when more than about 75% of the cells were cornified. Stage II was considered as beginning when approximately half the cells were nucleated pavement cells, and stage III when leucocytes had reappeared. It is agreed with Selle ('22) that Stockard and Papanicolaou's stage IV probably is an artifact and should be disregarded. Our departures, then, from the terminology employed by Stockard and Papanicolaou are the subdivision of their stage I into stages Ia, Ib, early, middle and late, and the omission of their stage IV.

In the collection of material indispensable assistance has been given by Messrs. R. J. Blandau, J. L. Boling, E. W. Dempsey, C. W. Hagquist and D. B. Jennison, and Drs. R. Hertz and H. I. Myers to all of whom I am deeply indebted. In addition, Mr. Dempsey has given valuable help in the statistical treatment and review of certain of the data. Acknowledgment is also made to the Winthrop Chemical Company for its generous contribution of antophysin.

OBSERVATIONS

The data obtained when smears were made within ½ hour after the beginning of heat and within ½ hour after the end of heat are summarized in table 1. When the differences between the vaginal smear types represented in this group are recalled, the lack of relationship between them and heat will be readily apparent. Before the first cornified cells had appeared about 5% of the animals had gone out of heat and sometime during this stage about 25% came into heat. Sometime during stage Ib, early, that is before more than 25% of the cells had become cornified, approximately 60% of the animals examined were in heat. Similarly, sometime during stage Ib, middle, about 60% of the animals were in heat, although when this stage began as many as 35% had gone out of heat. When Ib, late, began approximately 80% of the animals had gone out of heat. The remaining 15% to 20% were in heat sometime during this latter stage.

TABLE 1

Relationship between the vaginal condition and heat when smears were made at the beginning and end of heat

'IN' SMEAR	'OUT' SMEAR	TOTAL IN EACH GROUP	AVERAGE LENGTH OF HEAT
Prior to Ia	Prior to Ia	1	
Prior to Ia	Ia	2	
Ia	Ia	59	9.31 hours (range 1 hour to 20 hours)
Ia	Ib, early	138	
Ia	Ib, middle	72	
Ia	Ib, late	0	
Ib, early	Ib, early	115	8.38 hours (range 2 hours to 20 hours)
Ib, early	Ib, middle	245	
Ib, early	Ib, late	8	
Ib, early	II	0	
Ib, middle	Ib, middle	171	7.53 hours (range 1 hour to 14 hours)
Ib, middle	Ib, late	84	
Ib, middle	II	11	
Ib, middle	III	0	
Ib, late	Ib, late	41	6.56 hours (range 3 hours to 11 hours)
Ib, late	II	10	
Ib, late	III	3	
II	II	0	
II	III	1	A 7 hour heat
III	III	1	A 1 hour heat

It cannot be said, therefore, that with respect to the vaginal condition there is a definite heat time. To be sure, more animals are in heat sometime during the periods Ib early and Ib middle, when up to 75% of the cells are cornified, than at any other time. On the other hand, since the average length of heat is only 8 hours while the combined average lengths of these stages is about 18 hours, and the time of heat varies during even this period, its certain detection still depends upon frequent examinations for the copulatory response or the reaction to the male.

Also of interest is the relationship which often existed between the length of the preceding reproductive cycle and the vaginal condition at heat. In 141 animals that came into heat during stage Ia, the length of the preceding cycle averaged 16 days, 1 hour with a range from 13.5 to 21 days. In twenty-three animals that came into heat during stage Ib, late, the average length of the preceding cycle was 17 days, 17 hours with a range from 14.6 to 21 days. The variation is great in both groups. Nevertheless, the average difference of 40 hours when heat occurred late with respect to the vaginal condition is 5.84 times its probable error. This appears to indicate that when heat does not coincide with the first vaginal changes, the latter proceed normally and independently. The explanation may be found in the manner heat is produced and is discussed elsewhere (p. 322).

An unexpected relationship revealed by the first procedure is that between the length of heat and the time of its occurrence with respect to the vaginal condition (table 1). Not all animals that came into heat before or soon after the first cornified cells had appeared remained in heat longer than animals that came into heat later. The number that did, however, is sufficiently large to make the differences significant. Oestrus in animals that came into heat during stage Ia averaged 0.93 hour longer than in animals which came into heat during stage Ib, early, a difference that is 4.21 times its probable error. Oestrus in animals that came into heat during stage Ib, early, averaged 0.85 hour longer than in animals which came into heat during stage Ib, middle, a difference that is 6.53 times its probable error. Oestrus in animals that came into heat during stage Ib, middle, averaged 0.97 hour longer than in animals which came into heat during stage Ib, late, a difference that is 6.20 times its probable error.

The second procedure which involved making smears at half-hourly or hourly intervals from the time the vaginal membrane opened until the animals had been out of heat several hours gave rise to a complication for which allowance must be made in any discussion of the results.

In this group of cases the time of sexual receptivity was generally later with respect to the vaginal condition than it was when only two smears were made. Under the latter circumstance heat began and ended more frequently during stages Ia, Ib, early and Ib, middle (table 1). When smears were made at half-hourly or hourly intervals or a glass rod was inserted at half-hourly intervals, heat began and ended more frequently during stages Ib, middle and Ib, late (table 2). This could be attributed to an advancement of the vaginal changes in consequence of the frequent stimulation, the time of heat remaining constant, or to a delay in the time of heat, the time of the vaginal changes remaining constant. The former alternative appears to be more likely because the average length of the reproductive cycles preceding the period of heat when the smears were made was 16 days, 14 hours, an interval that is not significantly longer than that of 16 days, 7 hours for the three colonies.

The restriction of this effect to the time of the vaginal changes is difficult to account for unless it can be that the layers of cells are sloughed off more quickly in consequence of the frequent insertion of the medicine dropper or glass rod. Recognition of this effect, though, whatever its cause may be, is important for the review of the work which follows. If vaginal changes are accelerated in consequence of this procedure, the method is not the accurate means of determining the length of each stage it was hoped it would be. Presumably all stages are somewhat longer in normal animals than the averages compiled from the data in our possession indicate.

The duration of each subdivision of the old stage I varied greatly. Stage Ia varied from complete absence (apparently it can occur prior to rupture of the membrane) to 38 hours in length, the average for twenty-five animals being 11.6 hours. Stage Ib, early, varied from 1 to 28 hours, the average for twenty-five animals being 5.9 hours. Stage Ib, middle varied from 4 to 40 hours, the average for twenty-seven animals being 12.1 hours. Stage Ib, late, varied from 1½ to 34 hours, the average for twenty-four animals being 9.7 hours. When the data are recombined observations on twenty-three animals indicate that the average length of the old stage I is 39.5 hours and the range 21.5 to 61 hours, a length that is much greater than that given by Stockard and Papanicolaou.

TABLE 2

Relationship between the vaginal condition and heat when smears were made at half hourly or hourly intervals and following insertion of glass rod at half hourly intervals

'IN' SMEAR	'OUT' SMEAR	ANIMALS
Ia	Ia	0
Ia	Ib, early	1
Ia	Ib, middle	3
Ib, early	Ib, early	0
Ib, early	Ib, middle	5
Ib, early	Ib, late	0
Ib, middle	Ib, middle	8
Ib, middle	Ib, late	8
Ib, middle	II	1
Ib, late	Ib, late	12
Ib, late	II	1
Ib, late	III	0
II	II	0
III	III	2

Stage II was less variable and much shorter as others have noted. In twenty animals its length averaged 3.3 hours with a range from 1.5 to 7.5 hours. Since many observations were discontinued soon after stage III had begun, no effort was made to determine its length. In normal animals, however, lengths of 9.5 hours were recorded, and in three animals that did not come into heat lengths of 7, 10 and 18.5 hours respectively were recorded before the observations were discontinued.

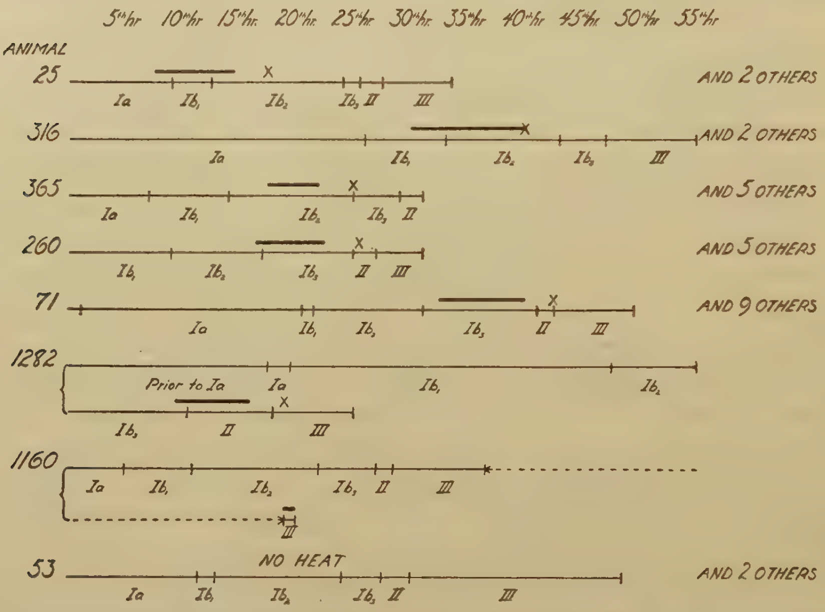

Fig. 1 Relationship between the vaginal condition and heat (heavy solid line) and the estimated time of ovulation (marked ×) when smears were made at half-hourly or hourly intervals. For brevity, Ib, early, middle and late are shown as Ib$_1$, $_2$ and $_3$, respectively. The dashes in the record of animal 1160 represent an interval when no smears were made.

The relationship between the vaginal condition and heat when smears were made at half-hourly or hourly intervals is shown in figure 1. That the relationship is no more constant in this group than in the first group can be seen immediately. Heat began before the cornified cells had appeared, it began when only a few had appeared, it began when most or all were cornified and it occurred during stage III after the pavement cells and leucocytes had appeared.

In addition, the procedure of making frequent smears enabled us to confirm what Ishii ('22) and Hemmingsen ('33) have found for the rat, what Fellner ('32) has found for the guinea pig, and what Grant ('34) has found for the ewe, namely that vaginal changes can occur in normal sequence in the absence of heat.

A last contribution from this procedure concerns the question of the identification of the various stages when leucocytes are present. Usually they had disappeared when the membrane opened and the identification of stage Ia presented no problem. In instances, however, many still were present and perhaps such a smear should have been classified unreservedly as prior to Ia. But normal behaving animals were found in which the leucocytes persisted into the cornified stages when they disappeared. In such cases the practice was followed of classifying the smears on the basis of the epithelial cell type rather than upon that of the presence of leucocytes. This policy has had the effect of keeping the data more in line with the older conceptions, but it may mean that the exact frequency with which animals came into heat prior to stage Ia has not been indicated.

Also of importance in the classification of smears was the fact that not infrequently leucocytes appeared in large numbers before pavement cells had begun to supersede the cornified cells. When this happened the sharp separation of stages Ib, late, II and III was difficult, but such smears were not classified as stage III until the rounded pavement cells were clearly separated by predominating numbers of leucocytes.

The importance of these points for one who is depending upon the smear for identification of heat is that the presence of leucocytes does not exclude the possibility of heat. Exclusive of animals that came into heat prior to Ia and during stage III, seven animals whose vaginal condition was noted every half hour or hour came into heat when leucocytes were present, and in not less than sixty-four cases leucocytes were present in single smears made within a half hour after the beginning of heat.

It will be realized from the material reviewed above that the occurrence of heat at any time beyond the limits of stages Ia, Ib, early, middle and late, and II is rare. Under conditions, though, it does occur, sometimes prior to stage Ia and sometimes during stage III. Its occurrence during stage III in two of the four cases encountered may have been the consequence of repeated vaginal stimulation because this latter group of animals numbered only forty-one whereas the other group in which two cases also were found numbered 968.

During the observations it was shown that heat can be produced experimentally prior to stage Ia. This was accomplished by the injection of antophysin on the thirteenth or fourteenth day of the cycle as noted above. In three animals the heat periods which lasted 4 to 6 hours occurred prior to stage Ia. In the fourth animal the heat period of 13.5 hours began prior to stage Ia, but ended during stage Ib, middle. The full significance of this result is not known unless it can be that a quantity of oestrin subminimal for the production of vaginal changes is sufficient to condition the animal for heat. The fact that adequate conditioning has occurred is shown by the appearance of heat at this time following the injection of progesterone (Dempsey, Hertz and Young, '36). When, therefore, antophysin is injected the follicular changes leading to heat progress so rapidly that the slower vaginal response cannot coincide in its occurrence. This suggestion which is admittedly at variance with current conceptions of oestrin threshold requirements is being investigated.

The observations reviewed in the preceding paragraphs are felt to demonstrate clearly that whereas heat and the vaginal changes ordinarily bear a close relationship one to the other, these two oestrous phenomena are separable. There remain data bearing upon the time of ovulation. Is it associated more closely with the vaginal condition and therefore, can the time of ovulation be determined from the vaginal condition; or is it associated more closely with the time of heat; or does the time of its occurrence vary independently with respect to the vaginal condition and the time of heat?

Information bearing upon this question has been obtained from a variety of observations and tends to support the second possibility. The close relationship which exists between the time of heat and ovulation has been discussed elsewhere (Myers, Young and Dempsey, '36). Because the relationship is so close and because the vaginal condition varies with respect to the time of heat, it was antcipated that the time of ovulation and the vaginal condition would vary accordingly. This has been found to be true (table 3). In thirty-three cases the relationship between the time of ovulation and the vaginal condition varied exactly as one would expect on the basis of the close association between ovulation and heat, and the varying relationship between heat and the vaginal condition in normal animals.

TABLE 3

The vaginal condition in animals in which rupture of at least one follicle had occurred

STAGE	ANIMALS
Ib, early	5
Ib, middle	18
Ib, late	8
II	2

Additional support for this conclusion comes from the few cases in which the relationship between the time of heat and the vaginal condition departed rather widely from the normal. When heat was produced experimentally on the thirteenth or fourteenth day of the cycle by injections of antophysin and ovulation occurred, the latter was associated with the end of heat rather than with any particular vaginal condition.

In one animal from which smears were made at hourly intervals, stage Ib, late, appears to have been abnormally prolonged over 83 hours at the end of which time a 5½ hour heat period occurred. When this animal was killed 1½ hours after the end of this heat normal follicles about ready to rupture were present.

TABLE 4

Vaginal changes during the 'split oestrus'

ANIMAL	TIME OF HEAT	LENGTH OF HEAT *hours*	VAGINAL CONDITION
293	In 11/29/33, 1.00 A.M.		Ib, early
	Out 11/29/33, 3.00 A.M.	2.0	Ib, early
	In 11/29/33, 4.00 A.M.		Ib, early
	Out 11/29/33, 9.30 A.M.	5.5	Ib, middle
922	In 10/13/35, 10.00 A.M.		Ia
	Out 10/13/35, 12.00 M.	2.0	Ia
	In 10/13/35, 4.00 P.M.		No smear
	Out 10/14/35, 4.00 A.M.	12.0	Ib, middle
989	In 12/18/35, 12.30 A.M.		Ia
	Out 12/18/35, 1.00 A.M.	0.5	Ia
	In 12/18/35, 4.30 A.M.		No smear
	Out 12/18/35, 4.30 P.M.	12.0	Ib, middle
1080	In 12/ 9/35, 4.00 A.M.		Ia
	Out 12/ 9/35, 7.00 A.M.	3.0	Ib, early
	In 12/ 9/35, 5.30 P.M.		Ib, late
	Out 12/10/35, 2.30 A.M.	9.0	II
1095	In 10/24/35, 7.00 A.M.		Ia
	Out 10/24/35, 8.30 A.M.	1.5	Ia
	In 10/24/35, 12.00 M.		No smear
	Out 10/24/35, 2.00 P.M.	2.0	Ib, early
	In 10/24/35, 3.00 P.M.		No smear
	Out 10/25/35, 4.00 A.M.	13.0	Ib, late
1236	In 12/12/35, 11.30 P.M.		Prior to Ia
	Out 12/13/35, 3.30 P.M.	16.0	Ib, middle
	In 12/14/35, 1.00 P.M.		III
	Out 12/14/35, 6.30 P.M.	5.5	III

Two animals from which smears were being made at half-hourly intervals came into heat after stage Ib, late, had begun. When laparotomies were performed 3½ and 4 hours respectively after the beginning of heat, ovulation had not yet occurred. The indications, therefore, are that in these animals in which the vaginal changes probably had been hastened by frequent stimulation, the time of ovulation had not been changed correspondingly.

It is regretted that the conditions under which the material was collected did not permit immediate study. Had this been possible the time of ovulation might have been determined in the few animals that came into heat during stage III. As it is, no inference may be made either way.

The last group of data concern vaginal changes during the course of the 'split oestrus.' They are of interest because they too indicate that within rather broad limits vaginal changes can proceed independently of the course of heat (table 4). Without exception the progress of the vaginal changes was normal despite the interrupted character of heat.

DISCUSSION

The first consideration in reviewing the observations given above is the extent to which they suggest a modification of currently held opinions concerning the relationship between vaginal changes and heat and the time of ovulation. The fact that vaginal changes vary with respect to the time of heat has been reported for the rat (Ishii, '22; Long and Evans, '22; and Hemmingsen, '33) and the ewe (Grant, '34), although no special significance was attached to the observation except by Ishii, Hemmingsen and Grant. Long and Evans also noted that ovulation varies somewhat with respect to the time of the vaginal changes. No one, however, seems to have presented evidence for any species indicating that when the time of heat varies with respect to a given vaginal condition, ovulation tends to remain associated with heat rather than with the vaginal condition.

The corollary to the above as far as the guinea pig is concerned is that neither the time of heat nor the time of ovulation can be determined accurately from the vaginal condition. If heat is to be detected with certainty and especially if information is to be had concerning its length and character, and if the time of ovulation is to be approximated more closely than it has been heretofore, dependence must be placed upon the behavioral response.

It is possible of course that the electrical method of Burr, Hill and Allen ('35) for determining the time of ovulation in the rabbit may be applicable to the guinea pig. If so, it will represent another distinct advance, but even then observations of behavior would be necessary in ascertaining the approach of ovulation.

Although they have been quoted to the contrary (Squier, '32) and the general opinion seems to have developed that heat is associated with cornification rather than with an earlier vaginal condition as well, our understanding is that Stockard and Papanicolaou regarded the early part of their stage I as the time of copulation. To the extent that animals very often are in heat during this period, they are correct, but the statement that copulation never is accomplished except at this time is not true. Genther ('34) likewise is essentially right in stating that the most favorable time for mating is during stage I (our stages Ia and Ib, early) when cornified cells have not yet appeared or are just appearing. If animals will not mate then, the chances are they soon will. But not even during these stages and stage Ib, middle, when heat is most likely to occur, can it safely be inferred from the vaginal smear picture that an animal will mate.

These departures from the generally held conceptions of the relationships between the vaginal condition, heat and the time of ovulation in the guinea pig require some explanation. The latter probably is to be found in the method of investigation that was employed which consisted of a comprehensive study of the various related events of reproduction in a single species observed continuously, day and night over relatively long periods of time. Quite obviously the value of such a method could not be appreciated until it was known from the fundamental early studies how ramified the subject is.

A second factor has been the ability to ascertain so accurately the time of heat. As long as sole dependence was placed upon the smear, a reasonably accurate determination of relationships between heat and the contemporary morphological events of the cycle was not possible. When, however, a more reliable method for determining the time of heat was found, it was not unnatural that some revisions would be made.

Different as the conclusions given above may be from prevailing opinions, there is nothing about them that is inconsistent with our knowledge of the ovarian changes at heat and the cause of heat in this species (Myers, Young and Dempsey, '36; and Dempsey, Hertz and Young, '36). In fact, one of the observations which led us to suspect that heat is not caused by the action of oestrin alone was that heat and the vaginal changes showed a varying relationship. If vaginal changes are caused by nothing more than the action of oestrin, the ease with which the time of heat shifts with respect to the vaginal changes suggested that some additional factor is involved in the production of heat. It has been postulated that this supplementary factor is progesterone produced in the still unruptured follicle although demonstration of this hormone in the follicle has not been made. In every other respect, though, results from the experimental work are sufficient to account for the varying relationships between the vaginal condition and heat and ovulation on the one hand, and the close relationship between heat and ovulation on the other.

Tentatively, it appears that the rising oestrin level, in consequence of follicular development during the late dioestrum, conditions the animal for heat and initiates the vaginal changes which proceed, as far as we know, without any further stimulus. The onset of heat, however, appears to be indirectly dependent upon the release of the luteinizing hormone from the hypophysis. The latter in turn causes the preovulatory swelling and ovulation (Foster and Hisaw, '35), and, according to our hypothesis, the concomitant production of progesterone which is directly responsible for heat. In such a scheme the time relationships of the vaginal changes to heat and ovulation would vary as the time of the release of the luteinizer varies. When release of this hormone occurs early, the interval from the beginning of the preceding heat would tend to be relatively short and heat and ovulation would coincide with the first vaginal changes such as stage Ia. When for some reason release of the luteinizer occurs later, the interval from the beginning of the preceding heat would tend to be relatively long and heat and ovulation would coincide with the later vaginal changes such as stage Ib, late. Both possibilities seem to have been realized in normal animals from which smears were examined (p. 311).

In a study of heat it is not sufficient to be able to produce it by means of replacement therapies. It is also important to know why heat is predominantly nocturnal, why its intensity is greater at the beginning than toward the end and why its length is so variable. Together these and other questions constitute what can be thought of as the regulatory aspects of the problem. For the last of these, the data reviewed above may make some contribution, although it is appreciated that the length of heat depends upon the interplay of many factors.

It has been known for some time (Young, Myers and Dempsey, '33) that the length of heat varies greatly from animal to animal and from one reproductive cycle to the next, but neither any explanation for such differences nor any means of investigating the cause has ever been suggested. One possibility, however, has developed from the observations bearing upon the vaginal condition and the length of heat.

It will be recalled that when heat occurs early with respect to the vaginal condition its average duration is significantly longer than when it occurs late. The only explanation suggested thus far is that an animal conditioned for heat can be thought of as being poised for heat, and that the conditioning is not of indefinite duration. If the heat stimulus occurs early when the conditioning is optimal, heat may be long. On the other hand, if the heat stimulus occurs late, the conditioning has passed beyond the peak of maximal effectiveness and heat tends to be short. The suggestion is speculative, but since both vaginal changes and heat can be produced by replacement therapies, investigation of the idea is practicable.

SUMMARY AND CONCLUSIONS

1. Vaginal smears were made at known times with respect to the time of heat and ovulation from guinea pigs under continuous, day and night observations over periods from 2 to 6 months duration. Before a satisfactory determination of the relationship between the vaginal changes and the time of heat and ovulation could be made, a subdivision of the old stage I was necessary. The new stages are Ia, composed of superficial cells only; Ib, early, when less than 25% of the cells are cornified; Ib, middle, when 25% to 75% of the cells are cornified; and Ib, late, when more than 75% of the cells are cornified. Stages II and III are no different from those described by Stockard and Papanicolaou. Their stage IV has been disregarded.

2. In normal females heat was detected only rarely prior to stage Ia and during stage III. On the other hand, sometime during stage Ia about 25% of the animals came into heat, sometime during each of stage Ib, early, and Ib, middle, about 60% of the animals were in heat, and sometime during stage Ib, late, 15% to 20% were in heat. It is also shown that vaginal changes can occur in normal sequence in the absence of heat, and that the presence of leucocytes does not exclude the possibility of heat. The conclusion, therefore, is that no vaginal smear picture is associated sufficiently definitely with heat to make this method completely satisfactory for determining the time of heat in this species. Certain detection of heat and information bearing upon the length and character of heat depend upon frequent examination for the copulatory response.

3. When smears were made at half-hourly or hourly intervals the time of heat generally was later with respect to the vaginal condition than it was when smears were made only at the beginning and end of heat. The evidence indicates that this changed relationship is attributable to the advancement

of the vaginal changes in consequence of frequent stimulation rather than to a delay in the time of heat.

4. In normal animals ovulation occurred during stages Ib, early, Ib, middle and Ib, late, when heat had occurred during these stages. From these and other data it is concluded that ovulation is associated with heat rather than with a given vaginal condition, and as a corollary, that the vaginal condition cannot be relied upon to indicate the time of ovulation.

5. When animals came into heat early with respect to the vaginal condition the average length of the reproductive cycle was less than when animals came into heat later. This has been taken to indicate that within fairly broad limits vaginal changes proceed independently of the time of the heat stimulus.

6. When a 'split oestrus' occurred vaginal changes proceeded normally despite the interrupted character of heat.

7. The data summarized above are believed to confirm the suggestion made previously that vaginal changes and heat are separable. This conclusion is shown to be reconcilable with recent experimental work on the cause of heat.

8. When animals came into heat early with respect to the vaginal condition the average length of heat was greater than when they came into heat late. The possible significance of this for the problem of the length of heat is discussed.

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EXPERIMENTAL STUDIES ON SPERMATOGENESIS IN THE HOUSE SPARROW, PASSER DOMESTICUS (LINNAEUS)[1]

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TWO TEXT FIGURES AND TWO PLATES (FIVE FIGURES)

INTRODUCTION

Investigators of avian spermatogenesis have been surprised at the scarcity of actively dividing cells in their preparations of adult bird testes. Although the enormous increase in the size of the testis of the house sparrow during March (Keck, '34) must be ascribed to a high mitotic activity, Foley ('28) and others had little success in finding mitotic figures in material fixed during the daytime throughout that month. After years of absolute failure to get 'a single mitotic figure,' Foley found "the mitotic phases of the first and second maturation divisions" in material collected in Louisiana in the month of March at 7.37 A.M. By 7.50 A.M. "few of the later phases" were found and there were no divisions observed during the remainder of the day. Later in the spring, no mitotic figures were found even as early as 5.30 A.M.

Etzold (1891) was the first to make a histological study of the development of the sparrow testis. He says that actual cell divisions are seldom found and attributes this to the probable rapidity of the process. Loisel ('01, '02) describes the spermatogonia of the sparrow as being derived from 'les cellules germinatives' by a process of amitosis and later multiplying by karyokinesis. However, he adds, "nous

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n'avons pu suivre toutes les phases de ces divisions.'' He also illustrates several division stages of the primary spermatocytes, including metaphase stages, but admits that his observations are insufficient on this subject. It seems apparent from the remarks of this author that there was indeed a scarcity of actively dividing cells in his material. Kirschbaum and Ringoen ('36) report having observed 'active mitotic divisions' in their preparations of sparrow testes but do not state the conditions under which these particular birds were kept prior to the fixation of the material. In view of the observations to be reported this would be of considerable interest.

The present work, as reported previously in a short abstract (Riley, '36 a), was undertaken to investigate more fully the possibility of a diurnal periodicity in the spermatogenic activity of the house sparrow and its controlling factors.

I wish to express my gratitude to Prof. Emil Witschi for suggesting this problem and for his helpful advice and criticism during the progress of the investigation.

MATERIAL AND METHODS

House sparrows were captured at night in barns around Iowa City. While in the laboratory the birds were in cages, 10 inches wide, 13 inches high and about 20 inches long. Such cages nicely accommodate four or five birds. The diet consisted of cracked corn.

To study spermatogenesis during the quiescent phase of the sex cycle, birds were stimulated by exposure to lengthened daily light periods (method of Bissonnette). A positive response can be recognized by a deepening of the pigmentation of the bill (Witschi and Woods, '36) or may be observed more directly by measurement of testis size in exploratory laparotomies. The treatment consisted of exposure to additional daily periods of light provided by a 100 watt incandescent bulb placed a few feet in front of the cage. The light period was increased 15 to 30 minutes daily, up to a total of about 4½ additional hours of light per day.

In the course of the experiments the importance of the temperature factor was discovered. Therefore it became a regular part of the technical routine to take the body temperature of every bird from which material was to be preserved for cytological study. The thermometer employed is a B-D Medical Center Security short-bulbed clinical thermometer. It is graduated by 0.2° to 110°F. and has a maximum diameter of 5 mm. The thermometer is moistened for purposes of lubrication and then projected down the oesophagus until the bulb rests within the region of the thoracic cavity. The handling and mechanical irritation involved in such procedure must necessarily excite, to some degree, such an active bird as the sparrow. However, every caution has been taken to keep the bird as quiet as possible. When the bulb of the thermometer is advanced it is often grasped by the bird voluntarily. By placing the bird on its back in the observer's hand and tipping its head back, the thermometer slides down the oesophagus with surprising ease. Once this has been accomplished, the bird lies quietly, in most cases without any necessary restraining force. When the temperature is determined during the dark period, care is taken to introduce only sufficient light for locating the bird. A shielded flashlight was used for this purpose.

Bouin's fixative has been employed most generally throughout the work. The dehydration technic suggested by Painter ('22) proved most satisfactory. The only deviation from this author's procedure was the substitution of oil of bergamot for oil of wintergreen. This technic avoids the hardening effects of the higher alcohols.

SEASONAL AND DIURNAL CYCLES

It is interesting that at the end of the so-called Indian summer in November, the house sparrow in Iowa experiences a mock breeding season. The bill darkens and the testes show enlargement. With the advancement of the winter, however, the testes regress again to the typical fall and winter condition. During this short period of testicular activity, quite numerous spermatogonial mitoses were found in testes of four sparrows preserved between 2.30 A.M. and 5 A.M. (November, 1935).

The fact that a period of clear sunny days is capable of causing a precocious development of the bird testis during the late fall suggests that light has some influence on the spermatogenic cycle.

That light can affect the reproductive cycle of some birds has been demonstrated repeatedly since the initial work of Rowan ('31) on the junco and of Bissonnette ('31) on the European starling. All these investigations show that in male birds exposed to additional daily periods of light, during the winter months, there is an accelerated development of the testis. The sparrow testis is likewise responsive to lengthened daily light periods as has been demonstrated experimentally by Kirschbaum and Ringoen ('36) and by the author (Riley, '36 b). Even though we have established that the testes of adult males do not respond to increased illumination in early fall, and Kirschbaum and Ringoen have found that decreased illumination is not always effective in suppressing testicular growth in the spring, there is no doubt but that increased light periods greatly stimulate the progress of seasonal gonadic development while the sparrow is in its responsive condition. It is all the more surprising, therefore, that no mitotic activity is found during the daytime with the exception of the early morning hours (fig. 4).

The normal diurnal cycle

Since it seemed probable that spermatogenesis is restricted to some period during the night, sparrow testes were preserved at intervals throughout the 24 hours of the day. Table 1 represents the results of collections made in the month of March, when the sparrow testis is highly active. It contains only material which was taken from birds captured the evening before.

As shown in table 1, mitotic figures are found during a great part of the dark period beginning about 4 hours after nightfall. The period of greatest activity lies between 2 A.M.

and 4 A.M. That occasionally a bird with low activity is found even at this hour is not surprising in view of the fact that the personal histories and the age of these newly caught specimens are unknown. The high spermatogenic activity at this time is well illustrated in figures 3, 5 and 6. Our table indicates the extent of mitotic activity only in general terms with one to three + signs. In view of constant changes in gonad and tubule size as well as the unequal distribution of active phases in different tubules, a more exact statistical representation of mitotic activity meets with great difficulties and has not been attempted here. The recorded observations

TABLE 1

Frequency of mitotic activity in the testes of thirty-two sparrows in March

NIGHT OF COLLECTION	NUMBER OF BIRDS	TIME OF FIXATION	DEGREE OF MITOTIC ACTIVITY
3/12/34	1	10.30 P.M.	—
3/12/34	1	10.30 P.M.	+
3/27/35	2	11.00 P.M.	—
3/27/35	1	11.00 P.M.	+
3/ 9/36	2	12.30 A.M.	+
3/ 9/36	1	1.30 A.M.	+
3/ 4/36	2	1.45 A.M.	+++
3/ 9/36	1	2.00 A.M.	++
3/28/35	1	2.00 A.M.	+++
3/28/35	1	2.00 A.M.	+
3/28/35	1	2.00 A.M.	++
3/ 9/36	1	2.15 A.M.	+++
3/ 4/36	1	2.45 A.M.	++
3/ 4/36	1	3.15 A.M.	+++
3/ 4/36	1	3.30 A.M.	+
3/ 4/36	1	4.00 A.M.	+++
3/28/35	2	4.00 A.M.	+++
3/ 4/36	2	5.15 A.M.	+
3/28/35	2	7.30 A.M.	—
3/28/35	2	10.00 A.M.	—
3/28/35	2	1.00 P.M.	—
3/28/35	1	4.00 P.M.	—
3/28/35	2	7.00 P.M.	—

A — sign indicates total absence of mitotic figures; the number of + signs the relative frequency of mitotic stages.

+, 1–150 mitotic figures per 0.1 mm.^{2} of sections 10 μ thick.

++, 151–300 mitotic figures per 0.1 mm.^{2} of sections 10 μ thick.

+++, 301–1000 mitotic figures per 0.1 mm.^{2} of sections 10 μ thick.

leave no doubt as to the existence of a definite diurnal spermatogenic cycle.

Control of the seasonal cycle by light

The effect of light on sexual activity in the male English sparrow has been described by the author ('36 b). When male sparrows were exposed to additional daily illumination beginning November 18, 1934, there was a marked response by the testis. At the termination of the experiment (December 28th) the testes were enlarged and bundles of spermatozoa were present in the seminiferous tubules. They exhibited, therefore, a stage of testicular development which normally is reached only during the month of March.

When male sparrows were exposed to lengthened days at a time more removed from the normal breeding season the result was quite different. In one experiment with light treatment starting September 30th only the testes of juvenile males responded. Adult males (those that had already passed through one or more breeding seasons) showed no response to increased illumination at this time. It is apparent that, within certain limits, light does affect the gonadal development of the sparrow. However, the unresponsiveness of the adult males early in the fall indicates also the presence of internal factors which help to determine the sequence of different phases in the breeding cycle.

The effect of light on spermatogenic activity late in winter is summarized in table 2. The birds used in this experiment were brought into the laboratory on January 22nd. To insure a complete state of quiescence before starting the experiment, the birds were kept under standard laboratory conditions for 16 days. Throughout this time, they received approximately 9½ hours of light daily. On February 7th laparotomies were performed to measure the size of the left testes. All were found to be very small, averaging 2.4 × 1.7 mm. From this time on the light period was increased 30 minutes per day.[2]

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[2] The length of the light period on the eighth day was the same as on the preceding day, but on the tenth day an additional hour of light was given instead of the usual ½ hour.

The testes from all birds exposed to increased daily illumination for more than 6 days show a definite increase in size and since they were preserved in the early morning, mitotic figures are plentiful in every case.

Control of the daily cycle by temperature

The restriction of spermatogenic activity to the night suggests that it may be associated with or controlled by some physiological change experienced by the bird with the transition from light to darkness. A marked lowering of the body temperature of the bird during the night is both striking and suggestive. Among the more recent students of the temperature of birds are Hilden and Stenbäck ('16), Wetmore ('21) and Baldwin and Kendeigh ('32). The first two authors reported normal daily variations in the body temperature of several species of large birds such as the hawk, domestic fowl, dove and owl.

TABLE 2

Effect of increased light periods on sparrows, February, 1935

BIRD NO.	DAYS OF EXPOSURE	INCREASE IN SIZE OF L. TESTIS	TIME OF FIXATION	DEGREE OF ACTIVITY
		mm.		
18	4	0.0×0.0	4.00 A.M.	—
20	5	0.2×0.2	4.00 A.M.	+
19	6	0.2×0.0	4.30 A.M.	—
22	7	0.5×0.5	4.20 A.M.	+++
16	9	0.8×1.2	4.00 A.M.	++
17	10	0.8×0.9	4.00 A.M.	++
21	11	1.0×0.6	4.00 A.M.	+++
27	12	1.6×1.0	4.00 A.M.	+++

The temperatures of the diurnal birds remain high throughout the day and drop quite suddenly with the coming of darkness. When Hilden and Stenbäck reversed the light period, reversed temperature curves were obtained. The elaborate and careful work of Baldwin and Kendeigh illustrates most impressively the high temperature of the day as compared with the low temperature throughout the night.

Although the present study of the temperature of the sparrow was made only with the aid of a clinical thermometer, which may appear rather crude as compared with the elaborate technical equipment of Baldwin and Kendeigh, it was found that our readings give essentially the same picture of temperature variation in the sparrow as that expressed in their curves for several species of Passeriformes.

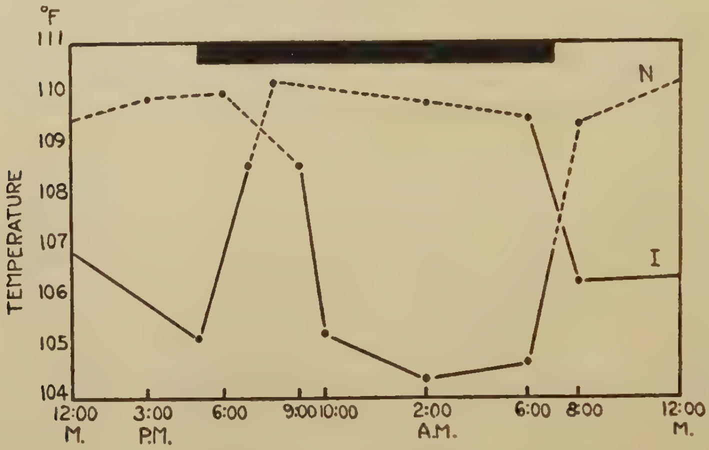

Fig. 1 Average daily variations in the temperature of the house sparrow, December, 1935. N, curve for birds with normal sequence of light and dark periods; I, curve for birds with an inverted light period. A continuous line represents the experimental dark period; a broken line, the experimental light period, and a solid bar, the length of the astronomical dark period.

The birds represented in the above curves (fig. 1) had been in the laboratory approximately 2 weeks previous to the day when the temperature readings were made. Exposure to a lengthened daily period accounts for the maintenance of a high temperature until 9 P.M. The maximum temperature recorded here lies just between the maxima recorded for the male house sparrow by Baldwin and Kendeigh (111.1°F.) and by Wetmore (108.6°F.). Individual temperatures as high as that recorded by the former authors have also been found by the present writer.

Evidence will be presented proving that the nightly lowering of the temperature is a necessary condition but not the stimulating cause for spermatogenic activity. This is already indicated by the fact that the diurnal temperature cycle is the same in and out of the breeding season. Even castrates give the same picture (fig. 2). In this connection we may also refer to work of Rowan (loc. cit.) and others showing that the seasonal cycle of wild birds of our north temperate zone is not controlled by temperature.

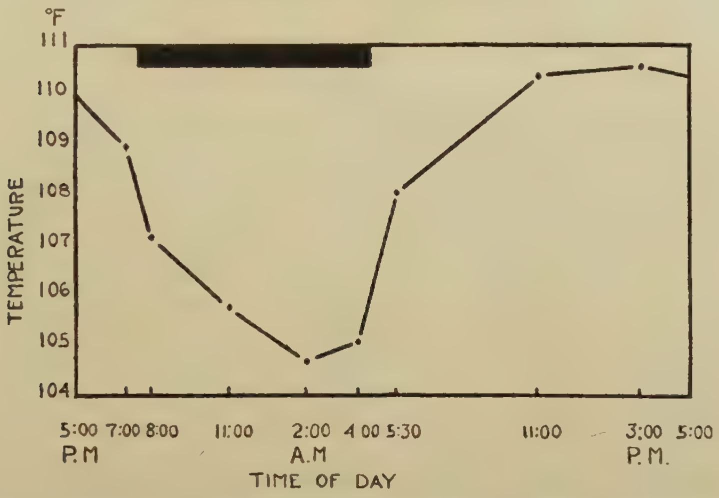

Fig. 2 Average daily variations in the temperature of castrate male sparrows. A solid bar represents the duration of the dark period.

Effect of inversion of the daily light period

If the daily light period is inverted (light from 7 P.M. to 6 A.M. and dark from 6 A.M. to 7 P.M.), the temperature curve is just the reverse of the normal one (fig. 1). If sparrows are exposed to light stimulation just before the inversion experiment is begun or the inverted light period is gradually increased so that the testes are in an active condition, mitotic figures appear in the late afternoon (fig. 7).

Birds I-92, 94 and 95 (table 3) were brought into the laboratory on November 16th. The daily period was inverted so that from 7 P.M. to 4.30 A.M. the birds were exposed to artificial light (100 watt bulb) and from 4.30 A.M. to 7 P.M. they were in darkness. The light period was increased approximately 15 minutes daily for 30 days. At the end of this time the birds were killed and the testes fixed at the hours stated in table 3. The four sparrows, I-1, 2, 3 and 4, had been exposed to increased daily light periods for 2 weeks previous to the light inversion experiment. The inverted light period extended from 7 P.M. to 11 A.M. The testes were fixed after the sparrows had been subjected to such experimental conditions for 6 days.

TABLE 3

Frequency of mitotic activity in birds with reversed light periods

BIRD NO.	TEMPERATURE	TIME OF FIXATION	MITOTIC ACTIVITY	SIZE OF LEFT TESTIS
	°*F.*			*mm.*
I-92	103.1	3.45 P.M.	+	3.9×2.6
I-1	102.6	4.00 P.M.	+++	4.2×3.3
I-4	103.3	4.00 P.M.	+++	4.1×3.2
I-94	104.4	4.15 P.M.	++	4.3×3.4
I-2	103.2	4.15 P.M.	++	4.3×3.4
I-95	104.0	4.30 P.M.	+	2.4×2.0
I-3	103.1	6.15 P.M.	+	3.9×3.0

As summarized in table 3 spermatogenic activity is now observed between 3.45 P.M. and 6.15 P.M. It is very probable that mitotic figures occurred even earlier than 3.45 P.M. The birds were sleeping during the afternoon and their temperatures were low.

Effect of experimental depression of the body temperature

If mitotic activity occurs during the dark period because of the lowering of the body temperature, it should be possible to find such activity also in testes of birds with an artificially lowered temperature during the light period.

It was found possible to lower the temperature during the day by clipping the feathers and exposing the bird to a low external temperature. Care must be taken to avoid a fall of the temperature below a vital level. After the bird had been kept with a low body temperature for several hours, the testes were removed and fixed. In table 4 it is shown that lowering of the temperature is followed by mitotic activity in the testis. The body temperature of three of these birds had dropped suddenly to a sub-vital level just before being killed. The number of mitotic figures in any of the testes preserved following this treatment was rather low. This must be attributed to the short space of time for which the bird was kept with a lowered body temperature. With the exception of LT-52, it was not more than 3 hours. The exception, LT-52, had been kept low for 6 hours. It is also quite possible that the maximal effect would be obtained if the temperature were kept for a period of several hours at a constant low level between 103°F. and 104°F. The mere fact that mitotic activity in daytime is obtained by this treatment proves that low temperature is a necessary condition and that mitosis does not follow light exposure as a delayed reaction.

TABLE 4

Frequency of mitotic activity in birds with artificially lowered temperatures in normal daylight

BIRD NO.	TEMPERATURE	TIME OF FIXATION	MITOTIC ACTIVITY	SIZE OF LEFT TESTIS
	°F.			*mm.*
LT-52	93.6	4.45 P.M.	+	3.8×2.9
LT-53	103.9	2.00 P.M.	+	3.5×2.6
LT-54	103.8	10.20 A.M.	+	3.0×2.1
LT-67	97.0	2.30 P.M.	+	4.0×2.5
LT-79	93.0	4.30 P.M.	+	2.9×2.1

Effect of high body temperature during the dark period

If a low body temperature during the day results in spermatogenic activity, it is reasonable to expect high temperature at night to suppress activity. It was decided to subject birds to enforced activity during the dark period. This necessitated an apparatus that would insure activity and at the same time protect the birds from injury. A slowly revolving cage, 1 foot in diameter, was found helpful, though we were not successful in keeping sparrows active in darkness for more than 5 hours without death resulting. Consequently, this experiment cannot be expected to give a fully satisfactory answer to the question raised.

The birds used in the experiment had been stimulated with light until there was definite evidence of testicular growth. At 10.30 P.M. on the night of our experiment, the daily light period was terminated. The birds were then left undisturbed in darkness and permitted to sleep until 1.30 A.M. At this time three of the birds were placed in the revolving cages. The birds were usually carried part way around the cage before dropping to the bottom for the process to be repeated over again. As a result of this constant disturbance, the temperature, which during sleep had been lowered, raised to a level approximating that of the daily temperature (table 5).

TABLE 5

Frequency of mitotic activity in testes of birds with enforced high temperature

BIRD NO.	TEMPERATURE	TIME OF FIXATION	MITOTIC ACTIVITY	SIZE OF LEFT TESTIS
	°F.			*mm.*
HTC-1 (control)	103.3	4.05 A.M.	+++	3.0×1.9
HTC-2 (control)	103.1	5.30 A.M.	+++	3.8×2.5
HT-56	110.6	5.25 A.M.	+	3.0×1.8
HT-57	108.0	5.40 A.M.	++	4.0×3.0
HT-58	106.5	4.15 A.M.	+	2.8×2.0

That some mitotic figures were found in the testes of the experimental birds is not surprising in view of the fact that 3 hours of rest in darkness intervened between the end of the light period and the start of enforced activity. Despite the relatively short period of enforced activity, the cytological pictures of the testes of the experimental birds showed fewer mitotic figures than the testes of the control birds. The evidence indicates that activity and consequent high body temperature inhibit spermatogenic activity during the dark period.

Effect of injection of gonadotropic hormones

Pregnant mare serum, when injected into male sparrows, causes a rapid development of the testis. In January, 1936, a group of male sparrows were injected daily with 0.2 cc. of pregnant mare serum[3] for 17 days. The final injection was given at 9 A.M. The object of the experiment was to determine how soon after the last injection spermatogenic activity would begin. It was with some surprise that little activity was noticed during the day following the injection of a dose which causes a rapid growth of the testis (table 6).

TABLE 6

Activity in sparrows injected with pregnant mare serum

BIRD NO.	TEMPERATURE	TIME OF FIXATION	MITOTIC ACTIVITY	ORIGINAL SIZE OF L. TESTIS	FINAL SIZE OF L. TESTIS
	°F.			mm.	mm.
64	111.2	11.00 A.M.	—	2.3×1.8	8.8×6.2
77	108.6	11.15 A.M.	—	2.0×1.7	8.4×6.2
67	110.4	11.30 A.M.	—	3.3×2.0	9.4×6.0
66	109.8	12.00 M.	—	2.0×1.7	6.9×5.5
70	109.8	12.10 P.M.	—	2.2×1.7	8.5×6.3
69[1]	110.6	1.00 P.M.	—	2.3×1.8	10.0×7.6
68	107.7	1.10 P.M.	—	2.0×1.5	8.0×6.5
73[1]	110.0	1.25 P.M.	—	1.9×1.4	6.5×4.5
76	109.4	1.30 P.M.	—	1.8×1.4	7.2×5.0
65	109.7	2.00 P.M.	+	2.3×1.7	7.5×5.6
60	110.5	3.00 P.M.	+	2.2×1.5	9.5×7.0
71	109.8	3.30 P.M.	—	1.5×1.0	7.3×6.0
72	103.6	2.00 A.M.	++	2.4×1.5	7.3×5.4
75	106.8	2.15 A.M.	++	2.4×1.8	8.2×5.8
74	103.4	3.00 A.M.	+	1.9×1.2	6.2×4.0
59	105.3	3.30 A.M.	—	2.1×1.2	5.0×4.3

[1] Birds that were not injected on the last day of the experiment.

A few figures were found in the testes of two birds preserved between 5 and 6 hours after the injection of the serum. On the other hand, the testes of birds 72, 75 and 74 showed activity in the early hours of the following morning in spite of the relatively long interval which had elapsed since the last injection of the hormone. It is not possible that the rapid increase in testis size, as shown by a comparison of the original size of the testis with the final size, can be accounted for by the little activity which occurs during the day. Apparently, most of the development takes place during the night.

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[3] Two-tenths cubic centimeter of this pregnant mare serum was equivalent to 2 rat units.

To show that the results were not due to the testes of the birds becoming immunized to the gonadotropic hormone, five newly caught sparrows were given the same dosage (0.2 cc.) for only 3 days. The testes were fixed between 5½ and 6 hours after a 9.00 A.M. injection, as before. In three out of the five cases, a few mitotic figures were found. The results indicate that even in the presence of large quantities of gonadotropic hormone spermatogenic activity is depressed during the day and awaits the more favorable physiological conditions of the dark period.

DISCUSSION

This study leads to the conclusion that spermatogenic activity in the sparrow is controlled by a complex of internal and environmental factors. Sexual activity is fundamentally dependent upon a relationship between the germ cells and a gonadotropic hormone produced by the pituitary. This relationship in the English sparrow has been well demonstrated by the experiments of Witschi and Keck ('35). A direct demonstration of the role of the hypophysis has been given by Benoit ('35), who found that the hypophyses from ducks, artificially stimulated by light, when implanted into immature mice, caused a definite increase in the size of uterus and ovary.

The seasonal sexual periodicity of the sparrow and many other wild birds indicates that there is an annual cycle of pituitary activity. The question arises as to whether this cycle is an inherent phenomenon or controlled by some external factor (for discussion see Witschi, '35). The widespread occurrence of seasonal sexual activity has led numerous authors to assume that environmental factors play an all-important role in the control of the reproductive cycle. Evidence in support of this view has been drawn chiefly from experiments wherein sexual activity has been accelerated by prolonging the daily light period during the quiescent phase of the cycle. Since the original investigations by Rowan and Bissonnette, much literature has accumulated on this subject (Miyazaki, '34; Benoit, '35; Clark, Leonard and Bump, '36; Kirschbaum and Ringoen, '36; Bissonnette and Csech, '36).

Whetham ('33) correlated the records of egg production of hens along different latitudes throughout the world with the daylight curves for these same latitudes. It was concluded that the light supply is an important factor in the seasonal distribution of egg production. This author brings out an interesting observation from a review of experiments wherein hens were subjected to artificial illumination during the winter months. Increase in light ration during this period is followed by an increase in egg production. However, when the birds are on a constant light ration, even though the level is a high one (24 hours) the effect wears off. This indicates that it is differences in light ration which supply the stimulus to increased production and not the amount of the light ration.

Such work on the effect of light has been extended to annual breeding mammals following Bissonnette's discovery ('32) that with increased illumination in midwinter, ferrets come into full oestrus. Almost simultaneously, Baker and Ranson ('32) reported that voles can be brought into full oestrus during their normal anoestrus periods by increasing their daily exposure to light. Several authors have confirmed the effect of additional illumination upon the oestrous cycle of the ferret (Hill and Parkes, '33; Marshall and Bowden, '34).

In spite of their success in modifying the oestrous cycle of ferrets, Hill and Parkes favor the idea of an inherent rhythm. This opinion is based upon an experiment in which ferrets, kept in total darkness from the end of January onward, came into oestrus at about the usual time (Hill and Parkes, '34).

Young, Myers and Dempsey ('33) have called attention to the cyclic occurrence of oestrus in the female guinea pig.

Most of the animals observed (66.7%) came into heat between 6 P.M. and 6 A.M. When the animals were confined to a completely darkened room, they lost this tendency to come into oestrus during the night hours (Young, Myers, Dempsey and Jennison, '34). The authors conclude that the change from daylight to darkness is the factor which is responsible for this periodicity in the guinea pig. It was further concluded, however, that since the animals in darkness were normal in respect to the length of reproductive cycles and the duration of oestrus, exposure to light is not necessary for the existence and maintenance of the reproductive rhythm in the guinea pig.

The results reported here for the house sparrow are in agreement with the findings of the above mentioned authors insofar as the testis responds to light stimulation during the late fall and winter. However, it was further found that adult male sparrows did not respond when exposed to additional periods of light during the month of October. These results indicate that light stimulation is effective only if the bird is in a responsive condition. It was concluded that there exists an inherent breeding cycle, which, through environmental factors, is coupled with the seasons. Sexual activity, therefore, appears to be controlled by internal as well as by environmental factors.

It is surprising, in view of the results of these light experiments, that spermatogenic activity should occur during the period of darkness. The most probable explanation seems to be that light stimulates the hypophyseal activity but that spermatogenic activity can proceed only at the relatively low temperature which prevails in the bird at rest.

The results of the injection experiments, summarized in table 6, prove that some mitotic activity may appear at relatively high body temperatures if gonadotropic substances are present in abnormally high quantities. We have used a moderately potent mare serum, administering daily not more than about 2 rat units of gonadotropic hormone. It appears, therefore, that the normal sparrow has a hormone level which even at the height of the breeding season does not rise to that produced in our injection experiment.

The dependence of spermatogenic activity in the sparrow on a relatively low body temperature is of particular interest in view of the findings of Moore and others (literature reviewed by Moore, '32) on the temperature-regulating function of the scrotum in mammals. It has always been puzzling that in birds spermatogenesis should proceed under temperature conditions which in mammals cause sterility. It is felt that the findings of the present work bring the conditions essential for sperm production in birds more closely into line with what is known to be true for other animals.

SUMMARY AND CONCLUSIONS

1. The development of the testis of the house sparrow is accelerated during the late fall and winter by additional illumination.

2. Light stimulation is effective only if the bird is in a responsive condition. Early in the fall (October), adult sparrows do not respond to increased illumination.

3. There appears to exist an inherent breeding cycle, which, through environmental factors becomes coupled with the seasons.

4. The diurnal spermatogenic cycle of the house sparrow during the normal breeding season is described. Spermatogenic activity occurs during the dark period with greatest frequency of cell division between 2 A.M. and 4 A.M.

5. A study of the daily variations in the body temperature of the sparrow shows a marked lowering of the temperature during the dark period (average of 110°F. during day, 103° to 104°F. during night). This reduced temperature is shown to be an essential condition for spermatogenic activity.

6. When the daily light period is inverted, the body temperature of the bird is low (103°F. to 104°F.) during astronomical daytime. Correspondingly, spermatogenesis proceeds now during the afternoon hours.

7. A few mitotic figures are found in testes during the day if the body temperature of the bird is artificially lowered for several hours while the bird remains in full daylight.

8. Enforced exercise during part of the night, with consequent high temperature, retards spermatogenic activity.

9. When a gonadotropic hormone, in a quantity well above the threshold, is injected into sparrows at 9 A.M., few mitotic figures are found in testes fixed within 5 to 6 hours. There is reason to believe that the rapid development of the testis produced by injections of pregnant mare serum takes place mainly at night. Even in the presence of large quantities of an effective gonadotropic hormone, spermatogenic activity awaits the favorable physiological conditions of the dark period.

10. The annual sex cycle and the diurnal spermatogenic cycle are controlled by different sets of factors. The seasonal cycle is controlled by hypophyseal activity, which, in turn, may be stimulated by light. Spermatogenic activity, however, is confined to the dark period of the night since it can proceed only at a low body temperature.

11. It is suggested that the dependence of spermatogenic activity in birds upon a low body temperature is comparable to the necessity for a scrotal position of the testes in mammals.

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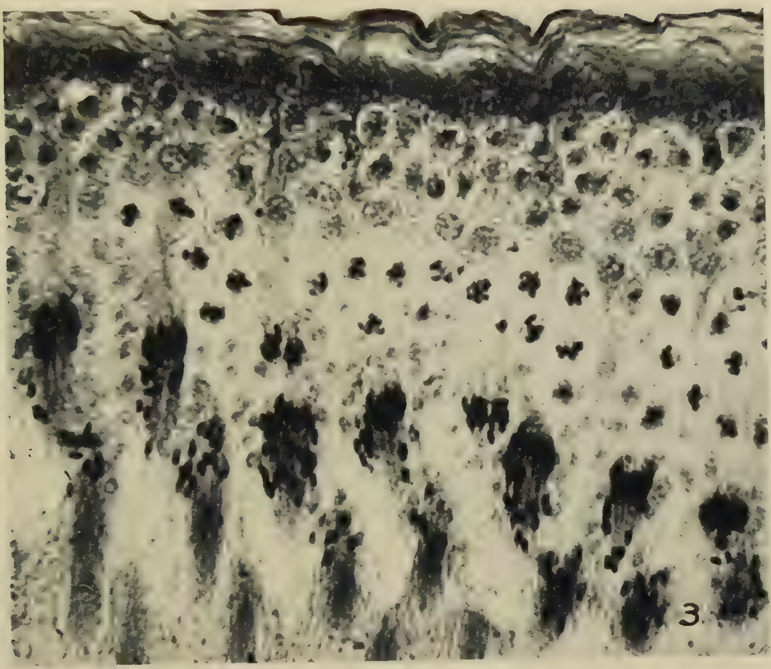
PLATES

PLATE 1

EXPLANATION OF FIGURES

3 Section of a testis preserved at 2 A.M. Numerous metaphases of primary spermatocyte divisions are present. A Sertoli cell can be seen to the left of center. × 550.

4 Section of a testis preserved at 1.30 P.M. In contrast to figure 3, there is not a single dividing cell present. × 550.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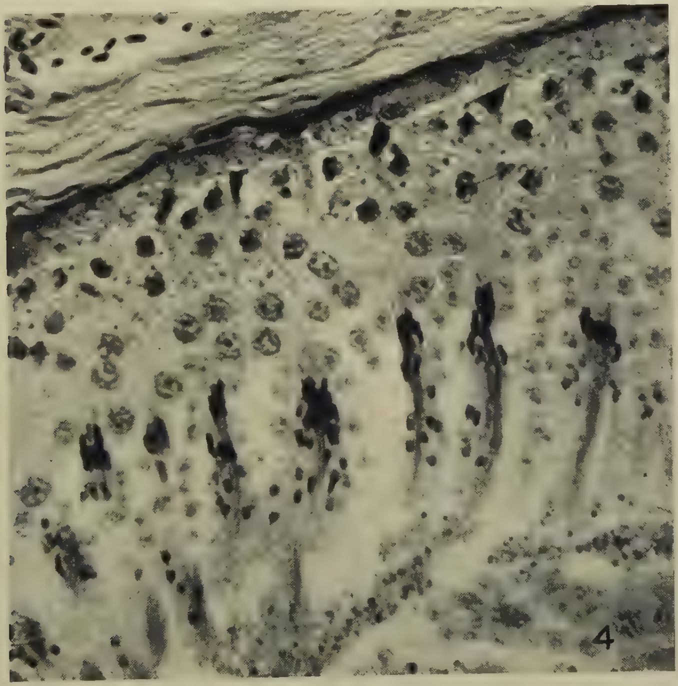
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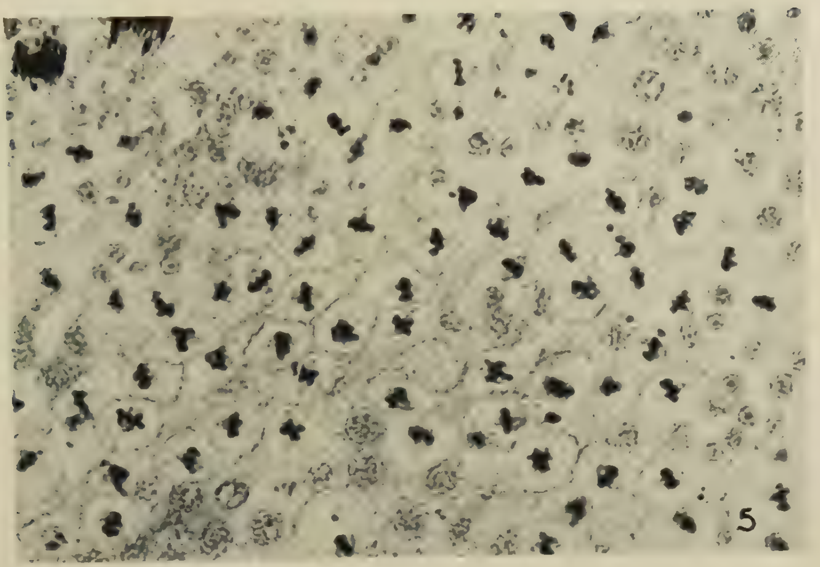
PLATE 2

EXPLANATION OF FIGURES

5 A field from a testis preserved at 2 A.M. showing many dividing primary spermatocytes. × 600.

6 Section of a testis preserved at 2 A.M. Near the periphery of the tubules are several metaphases of spermatogonial divisions; at the lower right are four metaphases of primary spermatocyte divisions. × 600.

7 Section of a testis preserved at 4 P.M. An area of actively dividing spermatogonia extends diagonally across the photomicrograph. × 650.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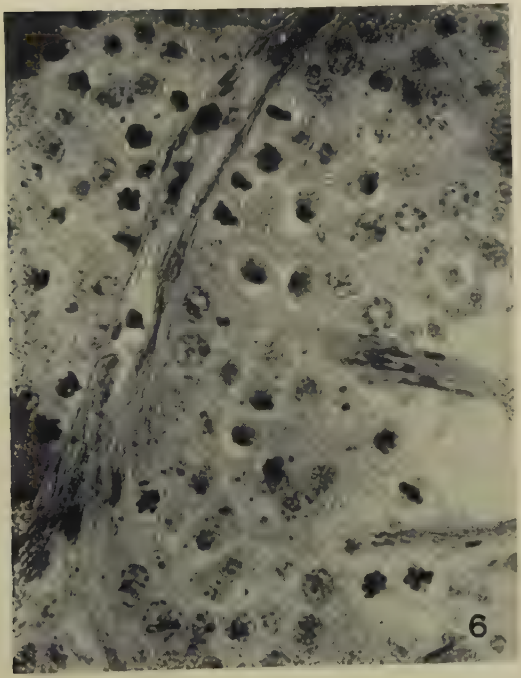
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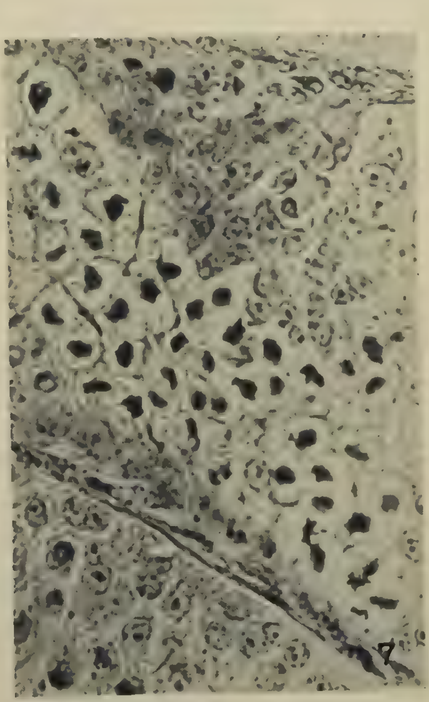
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HOMEOTRANSPLANTATION OF THE GUINEA PIG AND RABBIT ADRENAL GROWN IN VITRO

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SIX FIGURES

Transplantation of tissue, according to Loeb, is the complete or partial separation of a tissue or organ from its normal position and its transference to another position in the same or in another animal. When tissue is transplanted to other sites in the same individual, the transplanted tissue becomes an autotransplant; when transferred to another individual of the same species, it is a homeotransplant, and when transferred to an animal of another species it is called a heterotransplant. Autotransplantation of tissue or portions of organs is usually successful, if vascularity is established and provided the size of the graft is sufficiently small to permit of adequate nutrition. Accordingly, such grafts may remain alive, may grow and may function permanently. The reaction of the host toward a homeotransplant, however, differs considerably. Fibroblasts soon proliferate in the vicinity of the transplant; a dense fibrous zone often forms around it and a marked infiltration of the transplant by lymphocytes finally results in the complete destruction of the transplanted tissue. The reaction by the host, which is one that is typical of any reaction to a foreign body, is probably the result of differences in the biologic constitutions of the host and graft. The reaction of the host toward a heterotransplant is even more marked, but of the same order as that toward a homeotransplant, and within a short time the heterotransplant undergoes severe degenerative changes.

Since the antagonism arising between the host and the graft may be one of biologic incompatibility, recourse to the technic employed in tissue culture has proved of value in securing successful cross grafts in several instances. Stone, Owings and Gey ('33), who were the first to use this technic on mammalian tissue, showed that if fragments of the thyroid gland of a dog were grown in vitro for 30 days in the body fluid of another dog and then transplanted into the groin of the latter dog, the homeograft would be successful. It was their opinion that by growing the thyroid fragments in the plasma and serum of the host or recipient an adjustment of the graft to the tissues of the host had been made, and that the biologic differences between the thyroid glands of the two animals had thus been destroyed. Stone, Owings and Gey ('34) reported further experimental observations together with certain clinical observations in which such cross grafts had proved successful. Five of eleven dogs showed definite takes of thyroid grafts; in five cases in which thyroid grafts were made on human beings and in five cases in which parathyroid grafts were made, the interval following operation was insufficient to permit the results to be judged adequately, except in two cases. Parathyroid grafts were employed in these two cases; the grafts were functioning successfully in both of these cases, as judged by the health of the patients.

Following the pioneer work of Stone, Owings and Gey, Murray and Bradley reported the results of homeotransplantation of an adenoma of the islands of Langerhans into the axilla of a diabetic patient. Previous to the transplantation, the transplant was grown in the plasma and serum of the recipient for 3 weeks. Three months after the transplantation there was no change in the condition of the patient. Haymaker and Anderson ('36) cross-grafted the pituitary body of a rat into hypophysectomized rats after the pituitary body had been grown in the recipient's plasma and serum for 5 to 7 days. When an exploratory operation was performed, 45 to 109 days after transplantation, pituitary tissue could not be found although there was some lymphoid infiltration and proliferation of connective tissue. They concluded, however, that the pituitary body may be adapted for homeotransplantation because histologic repair of the testes, thyroid gland and adrenal glands of the hypophysectomized animals occurred and the normal weight of these animals had been restored after transplantation.

Selle transplanted cultured pancreatic tissue into dogs which had experimental diabetes. He cultured islet tissue from fetal animals and from animals whose pancreatic ducts had been ligated and he transplanted these cultures into the groin and axilla of diabetic dogs. These grafts were completely absorbed 3 weeks after transplantation.

Our report covers the results of a study we have made during the past year on the culture, growth and homeotransplantation of adrenal glands of newborn guinea pigs and rabbits. Transplantation has been made into the groin of adult rabbits in all instances. In our earlier culture of the adrenal glands, fragments of the adult gland were grown both in Carrel's flasks and in Maximow's slides. Since epithelium was more readily grown from fragments of adrenal glands of newborn animals than from fragments of the differentiated gland of adult animals we have restricted our later work to material from newborn animals.

METHOD

The adrenal glands were removed aseptically from litters of animals as soon after birth as possible. The glands were placed in warm Tyrode's solution and immediately fragmented into pieces approximately 0.5 mm. in diameter. Healthy adult, male rabbits, which previously had been selected and which had fasted, were bled by cardiac puncture; the plasma and serum were prepared. Six- or 7-day chick embryos were used to make the embryo extract. Equal parts of Tyrode's solution and serum were used in the preparation of the extract. D-5 Carrel flasks were used and fragments of the adrenal glands were placed on the clot, according to the technic described by King. The clot was prepared by adding 1 part of plasma to 3 parts of tissue extract. About twelve fragments of the adrenal gland were placed on the surface of the clot; we secured a more luxuriant growth when there were many fragments in a flask than we did when only a few fragments were placed in the flask. One cc. of the tissue extract was then added to the flask. This fluid was removed every 2 or 3 days during the period of growth and fresh tissue extract was added. All cultures were incubated at 37.5°C. for 2 weeks to 18 days. Subculturing was done as required, although there was but little if any liquefaction in the cultures. The fragments were then aseptically removed from the flasks and transplanted into the groin of the host from which the serum and plasma had been taken. About 80 to 100 fragments were transplanted in this way to each animal. Six animals comprise this series. Exploration of the site of transplantation was made at 2, 4, 6, 8 and 12 weeks after operation and portions of the tissue were removed for fixation and study.

OBSERVATIONS ON THE GROWING CULTURES

Observations were made daily on the character and extent of the growth of each fragment in each flask during the period of culture. No attempt was made to isolate cortical fragments from those taken from the medulla. In fragmenting the gland, bits of medullary tissue were often included with portions of the cortex; therefore, we are unable to say whether any given growth from a fragment is of cortical or of medullary origin. We are unable to specify the region in the cortex from which growth took origin in any given fragment. We were not particularly interested in the growth of any particular region. Our interest centered chiefly on the growth of the fragment in vitro and in its subsequent growth in the groin following transplantation.

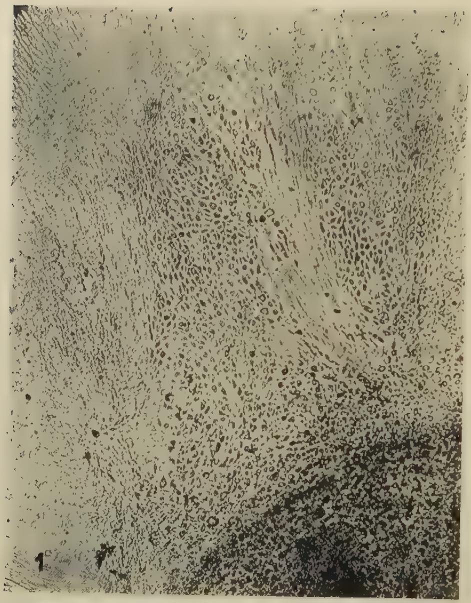
Growth began from most fragments after they had been incubated for 18 to 24 hours. In some instances growth was not seen until the second or third day, and in a few instances growth of cells never occurred. Fibroblasts appeared first, as a rule, although budding epithelial cells could be identified in regions in which the fibroblasts were not too dense. There seemed to be no difficulty in distinguishing epithelial cells from fibroblasts (fig. 1). Epithelium grows chiefly in the form of a pavement and consists of sheets of irregularly shaped cells with definite intercellular spaces. The fibroblasts, as a rule, were spindle shaped, but occasionally on the surface they assumed a pavement form not unlike epithelial cells. The growth of fibroblasts is syncytial and outlines of the cells were not readily discernible; the fibroblasts thus differ from epithelium, which has definite intercellular spaces. Macrophages wandered out from the explant on the third day; they proliferated rapidly and on the fifth day they were abundant.

The rate of growth of these three types of cells was exceedingly variable. In certain fragments the growth was restricted to certain regions, so that irregular patterns were produced. In many fragments the growth of fibroblasts exceeded the growth of epithelial cells and macrophages; although in other instances epithelial cells grew more rapidly and formed sheets of cells which extended beyond the zone of fibroblasts. The periphery of this epithelial sheet was usually irregular and formed finger-like processes of pavement-like growth.

Liquefaction occurred only occasionally and did not seem to depend on the growth of epithelium, which continued out over the surface of the clot during the 2 to 3 weeks of incubation. Where liquefaction did occur, growth subsided and irregularly shaped epithelial cells were freely dispersed throughout the medium.

HISTOLOGIC APPEARANCE OF FRAGMENTS FOLLOWING TRANSPLANTATION

Although these growing tissues were transplanted as discrete fragments there always was a tendency for them to coalesce at the site of the graft. When the growth was examined a week to 10 days after it had been transplanted, the



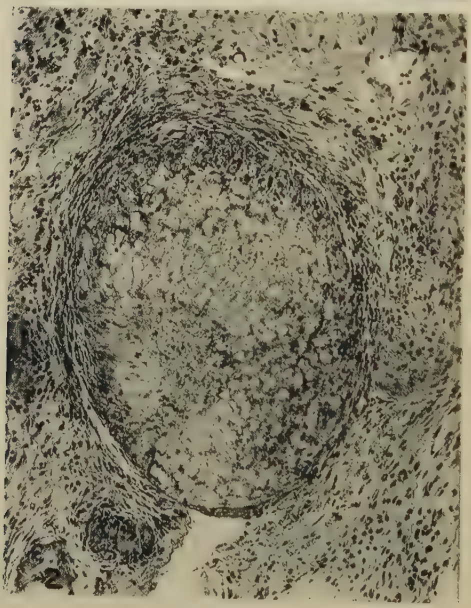
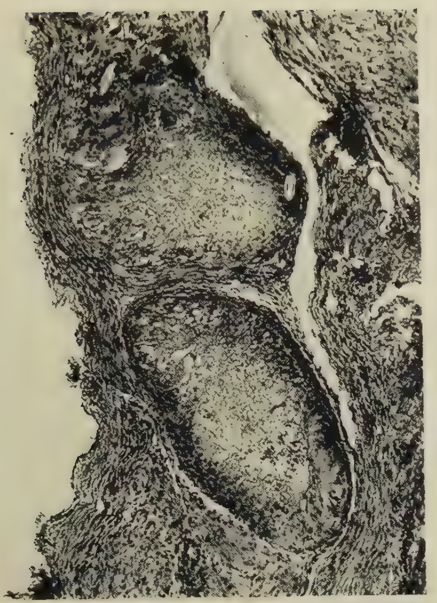


Fig. 1 Character of the growth of adrenal fragment of a newborn rabbit 10 days after explanting in a D-5 flask (× 65).

Fig. 2 Fragment of tissue removed from groin of adult rabbit 2 weeks after transplanting; one may note the fibrous band around fragment and the early infiltration with lymphocytes (stained with hematoxylin and eosin, × 125).



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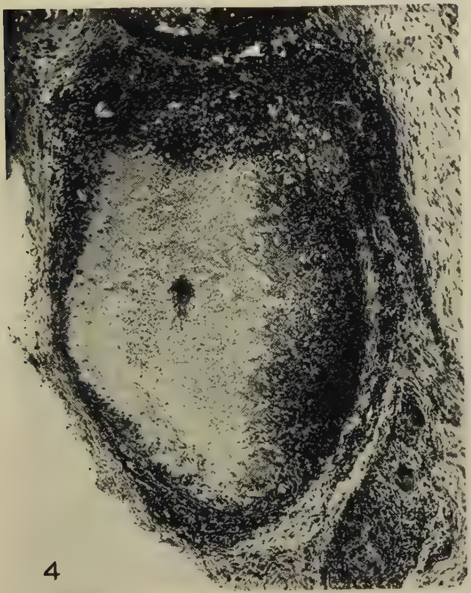

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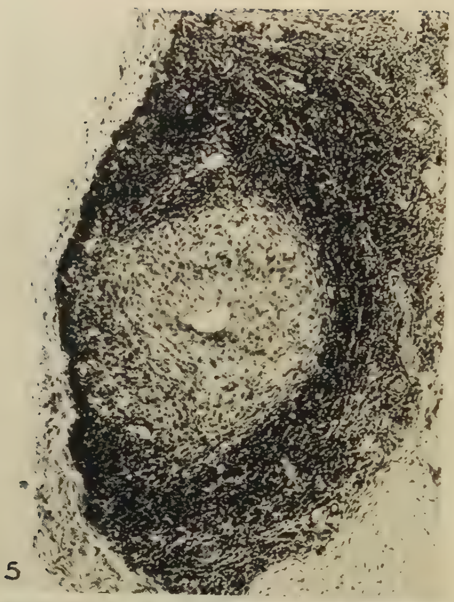
Fig. 3 Section of tissue removed from groin 4 weeks after transplanting; a slight increase in the infiltration of lymphocytes has occurred (stained with hematoxylin and eosin, × 80).

Fig. 4 Section removed from groin 6 weeks after transplanting; one may note further degeneration of fragment and increase in the size of the infiltrating zone of lymphocytes (stained with hematoxylin and eosin, × 70).



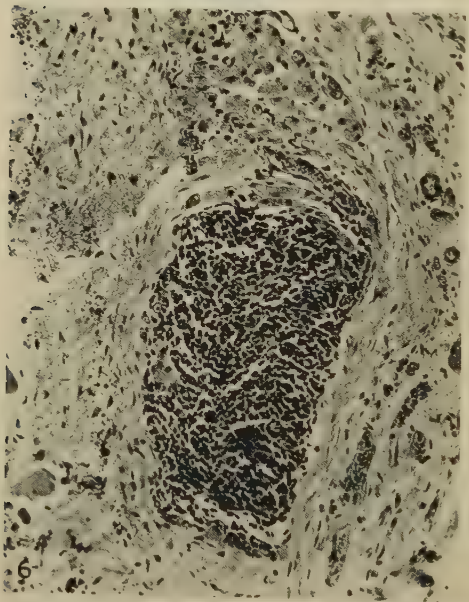


Fig. 5 Section of fragment removed from groin 8 weeks after transplanting, showing increase in infiltration of lymphocytes (stained with hematoxylin and eosin, × 95).

Fig. 6 Section of fragment removed 12 weeks after operation; fragment is completely overwhelmed with lymphocytes and histiocytes (stained with hematoxylin and eosin, × 165).

fragments had clumped together and had adhered by means of fibrous adhesions. When the animals were subjected to exploratory operations at varying intervals after transplantation, the fragments were always clumped and appeared as small, white, irregularly shaped bodies. Fibrous bands separated the fragments from each other but there was neither neutrophilic nor lymphocytic infiltration of the fragments during the first 10 days. Examination of fragments which were removed 2 weeks following operation (fig. 2), revealed a considerable amount of cellular infiltration. A fibrous band had walled off the fragment and within this band there were lymphocytes and histiocytes. Nuclear fragmentation and cytoplasmic degeneration of the adrenal fragment also was noted. Examination of sections of fragments removed 4 weeks following operation disclosed cytologic reactions which were similar to but more accentuated than those previously noted (fig. 3). As fragments were removed at successive intervals up to and including 12 weeks following operation, the extent of the lymphocytic infiltration became more marked (figs. 4, 5 and 6). Eight weeks after the operation, approximately half of each fragment was infiltrated with lymphocytes; 10 weeks after the operation, fully three-fourths of each fragment was infiltrated, and 12 weeks after operation the entire fragment was literally a mass of infiltrated cells. In addition to lymphocytes, sections of the fragments removed at 12 weeks contained many polymorphonuclear leukocytes and histiocytes. The fragments were usually well vascularized.

COMMENT

As a result of a study of the sections of adrenal fragments which were removed from the groin of rabbits at varying intervals after their transplantation, it seems clear that the grafts had not taken. They were walled off by fibrous tissue and there was local infiltration with lymphocytes, histiocytes and neutrophilic leukocytes. In general, the reaction by the host to these adrenal fragments was one that is somewhat characteristic of the reaction to any foreign body. Furthermore, the reaction somewhat resembled that which occurs after any homeotransplantation. Following the homeotransplant of cartilage or of portions of the thyroid gland, a thick layer of fibrous tissue soon forms around the transplant. Collections of lymphocytes and fibroblasts soon invade this fibrous band and penetrate the portions that have become necrotic. In the case of thyroid homeotransplants, fibroblasts of the host grow around the living acini, induce shrinkage, and finally cause complete obliteration of the acini (Loeb).

There are differences in the degree of reaction, however, on the part of the host, toward these adrenal fragments grown in vitro and toward an ordinary homeotransplant. When a thyroid homeotransplant is made, the extensive lymphocytic infiltration, according to Loeb, begins on the seventh or eighth day. Lymphocytes rapidly invade the transplanted tissue, overwhelm it, and assist in its destruction. Usually such a homeograft is completely destroyed in from 20 to 30 days after transplantation. In the case of these newborn adrenal fragments which were grown in vitro in the plasma and serum of the host, the destructive lymphocytic action is considerably deferred. The early reaction of the connective tissue, however, was similar to that which occurred when portions of the thyroid gland were used as homeografts; in both instances, a definite fibrous band formed around the transplant. It was not until the third week that any definite lymphocytic action was evident. Degeneration of the fragment continued and during successive weeks the degree of lymphocytic infiltration was increased; however, it was not until the tenth or twelfth week that complete infiltration of the graft had occurred.

It seems possible perhaps that we are dealing here with a graft which showed early indications of a successful take. Vascularity which develops in an autotransplant is always greater than that which develops in a homeotransplant. In these adrenal grafts, the periphery of the fragment was always highly vascular and small capillaries penetrated profusely through the capsule of fibrous tissue. This fact, together with the long deferred lymphocytic and histiocytic infiltration, is some evidence that the reaction here is not typical of that which occurs in the usual hemeotransplant. It is entirely possible that, in the pre-operative culture of these glands in the body fluids of the hosts, we induced a certain adaptability or adjustment of the fragments to the tissues of the hosts. The work of Stone, Owings and Gey ('34 a, b, c) has shown that such an adjustment is possible. Such an adaptation, or adjustment, if it did exist, was but temporary, for the degeneration which ensued and the accompanying cellular infiltration completely destroyed these grafts in 3 months.

There is no question as to the viability of the grafts we transplanted to the groin. That epithelial tissue was abundant is assured. We are not certain, of course, as to the particular zone of cortex which was growing or whether any medullary fragments were transplanted. The adrenal glands of the newborn animal are too small to permit any satisfactory identification of the pieces after the glands have been fragmented. Since a large number of living fragments were implanted, and since the groin with its capillary bed has proved so desirable a site for transplantation, the reasons for the degeneration are not clear. It is possible, of course, that biologic adjustment between the growing fragments and the tissues of the hosts may not have been made, so that we may have been dealing with an ordinary homeotransplant; however, the pathologic response to these 'in vitro' adrenal fragments did not occur in the same manner as that which usually occurs after homeotransplantation. To be sure, the same response did occur, but only after weeks had elapsed, and only after the fragments had ceased to live. May it not be true that these grafts were functional and successful, but that in the presence of adequate normal adrenal glands they ceased to function, underwent autolysis and were subsequently destroyed. The 'law of deficiency' first promulgated by Halsted has been questioned and there is little evidence to support the hypothesis that a need for a principle secures the successful graft of an organ or tissue elaborating that principle. However, Stone and his co-workers ('33), in their earlier experimental studies, removed two-thirds of the thyroid gland of the dog, in order to produce a deficiency, before they transplanted growing thyroid fragments to the groin. Haymaker and Anderson removed the pituitary bodies from rats in which they subsequently made pituitary homeotransplants. The grafts were considered successful inasmuch as the histologic damage occasioned by the hypophysectomy was repaired by the graft. This law of deficiency may well be tested by creating a loss of the principle through bilateral adrenalectomy just prior to transplanting fragments of adrenal glands to the groin. We have not done this on rabbits, but we are now studying the conditions in the rat with exactly these thoughts in mind.

SUMMARY AND CONCLUSIONS

This report covers a study of the transplantation of fragments of the adrenal glands of newborn guinea pigs and rabbits to the groin of adult rabbits in whose plasma and serum the glands had been grown. The adrenal glands were removed aseptically from newborn animals, fragmented and cultured in the plasma and serum of healthy adult rabbits. Extensive fibroblastic and epithelial growth occurred. After periods of growth ranging from 2 to 3 weeks, these fragments (usually 80 to 100) were aseptically transplanted to a rich vascular bed of the groin of the animals in whose fluids the cultures had been grown. These animals had not been adrenalectomized previously. At intervals ranging from 2 to 12 weeks, the groins were explored and fragments of tissue were removed for histologic study.

The following deductions have been made: 1) The adrenal gland of the newborn animal grows luxuriantly and rapidly; growing cultures were obtained and actively growing viable fragments were transplanted. 2) The initial reaction to the transplantation is a clumping of the fragments and the deposition by the host of a strong fibrous band around each fragment. 3) Two weeks after transplantation there is an initial infiltration of lymphocytes through the capsule to the periphery of the fragment. Degeneration of the fragment ensues, and the lymphocytic migration rapidly proceeds. Subsequently, polymorphonuclear leukocytes and histiocytes invade the fragment and 12 weeks after transplantation the transplant is completely destroyed.

The deferred lymphocytic and histiocytic reaction by the host differs from the reaction usually encountered after homeotransplantation and there are cytologic evidences that the graft may have been successful in the first few days following the operation.

The degree of vascularity of these grafts was greater than that usually seen in homeotransplants, and this, together with the delayed lymphocytic response, may indicate that some identity in the biologic constitution of graft and host had developed.

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THE THYROID GLAND OF THE NORMAL RAT: SIZE, DRY MATTER AND IODINE CONTENT[1]

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It has been shown (Remington and Levine, '36) that in growing rats reared on diets of varying degrees of iodine deficiency, definite and fairly quantitative relationships exist among the size, the iodine content and the dry matter content of the thyroid gland. Using uniform animals and a constant experimental period, it was found that these relationships can be expressed in curves in which thyroid weight (per 100 gm. body weight) is plotted against iodine content of the gland (per cent, dry basis) and against dry matter content. In order to make such relatonships more quantitative, and to extend them to rats differing in age, weight and sex, it is necessary to have more exact data than now exist as to the size, iodine content, and dry matter content of the thyroids of normal rats, reared on diets which are fully adequate in all respects, including iodine.

The first systematic study on the size of the rat thyroid in relation to age and weight is probably that of Jackson ('13). Combining the data of Jackson and those of The Wistar Institute, Hatai ('13) derived the following equation for expressing thyroid weight in terms of body weight:

$$\mathrm{TW(mg.)} = 0.0973(\mathrm{BW} + 27) + 13.9 \log(\mathrm{BW} + 27) - 22.6$$

Hatai was not able to detect any significant difference in the size of the thyroid due to sex. Freudenberger ('32), comparing rats of the Wistar strain with the Long-Evans hybrid

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strain, obtained values in good agreement with Hatai's curve, but found that the hooded rats had larger thyroids, at same ages, than the Wistar rats. Since they also made more rapid growth, the differences largely disappear when thyroid weight is expressed in terms of body weight. McCarrison ('30) determined thyroid weight on a series of rats from his colony at Coonoor, India, ranging in age from 20 to 150 days, and from 27 to 226 gm. in weight, and proposed the following equation (sexes combined)

$$TW(\text{mg.}) = 0.0609(BW-20) + 2.421 \log(BW-20) - 0.1153$$

The values for the Coonoor rats are almost exactly one-half those of Hatai. More recently (Hamilton, '34; Freudenberger and Billeter, '35) values about 40% lower than those of Freudenberger's earlier report have been found in the same colony. Hamilton attributed his lower values to differences in methods of dissection, but since the clean dissection of the thyroid gland is extremely easy and simple, such great differences do not seem likely. The only known difference in diet or handling was the addition of 1% of cod liver oil to the ration and the smaller glands have also been attributed to that addition (Freudenberger and Clausen, '35).

Within the last 4 or 5 years, as a measure of economy and convenience, many rat colonies in North America are fed commercial calf meals or dog foods, which universally contain both cod liver oil and iodized salt. Prior to that time, in rations mixed in the laboratory, little or no thought was given to satisfying the needs of the animals for iodine. For example, Freudenberger's earlier ('32) colony ration consisted of: Casein 15; whole milk powder 10; sodium chloride 0.8; calcium carbonate 1.5; unsalted butter 5.2; and whole wheat 67.5. This work was done in Minnesota, an area of known iodine deficiency, and hence dietary materials available would probably be deficient in this element. The writer has shown (Remington, Coulson and Levine, '36) that the goiter-preventing power of dried milk is in proportion to its iodine content. The same considerations may explain why several investigators have reported the iodine content of the rat thyroid as lower than that of pasture-fed animals or man.

In work done in this laboratory prior to 1933 (Levine, Remington and von Kolnitz, '33 a, '33 b), rats were reared on Russell's modification of the Sherman ration, which is as follows:

Dried meat and bone scraps	9.1
Dried whole milk	29.9
Ground whole wheat	59.8
Sodium chloride	1.2

The iodine content of this diet was found to range from 47 to 72 parts per billion, and thirty-nine 9-week-old rats to have thyroids (M and F) averaging 12.9 ± 0.25 mg. per 100 gm. body weight, with average iodine content of 0.0634%, dry basis; and we concluded that the diet was somewhat inadequate as to iodine, but not enough to give significantly enlarged glands. It will be noted that these values are intermediate between those of Hatai and of McCarrison (table 1).

TABLE 1

Weight of Normal Rat Thyroids

(As milligrams per 100 grams body weight)

According to Different Investigators

Body Weight grams	Hatai 1913 M & F	Freudenberger 1932				McCarrison 1930 M & F	Levine 1933 M & F	Hamilton 1934 M & F	Freudenberger 1935 F	Remington 1936 M & F
		Wistar Strain		Long-Evans Strain						
		M	F	M	F					
40	23.8	20.2	23.2	22.8	20.8	10.8				
70	20.3					10.0	16.4			9.7
100	19.0					9.4				9.4
130	17.7					8.8				11.0
150	17.3		16.2			8.6		10.4		10.9
170	16.6				16.7	8.4	12.9			9.1
200	16.0					8.2	10.9-F		9.8	7.6
240	14.7	15.9								7.6
250	14.4		12.9	18.9						6.8
280	14.3				14.5					7.5
340	14.3	12.1				9.6-M				7.6

In 1933 it became necessary for us to find a breeding ration that would be cheaper than one containing a considerable proportion of whole milk powder, and after a year of experimentation we adopted a commercial dog biscuit[3] which gave better growth and reproduction, and more uniform animals than the diet previously used. We have observed from time to time that animals reared on this ration have smaller glands of higher iodine content than those on the previous one. Histologically the acini of the thyroid are round or slightly ovoid in shape, well filled with dense homogeneous colloid, the epithelial cells being uniformly cubiodal, those of a single acinus being of constant height and regular arrangement. Occasionally a single acinus or group of acini can be found, usually near the periphery of the gland, which show vacuoles, or even a softening in the colloid. Some variation in degree of activity appears in different glands, but only rarely is there a softening of colloid in more than one or two acini in a given section. Furthermore, the unusual vigor and freedom from disease of our animals is evidence that the diet is an excellent one. According to the manufacturers, it contains both iodized salt and cod liver oil. Analyses in this laboratory revealed an iodine content of 80 γ per 1000 Calories, which is well above the minimal requirement of the rat (20 to 40 γ per 1000 Calories) as determined by us.

Our original stock came from The Wistar Institute, but later, through the courtesy of Dr. Victor C. Myers we obtained some hybrid animals from Western Reserve University, and through that of Dr. Arthur H. Smith some albinos from the colony at Yale University. These three stocks were kept separate until we had satisfied ourselves that under our handling no marked differences in thyroid development existed, after which they have been interbred at random for about 5 years. We have not noted marked variability in thyroid size of rats from different litters, when expressed in terms of body weight. Systematic data on thyroid size, iodine content, and dry matter content, in relation to age, weight and sex, of animals in this colony, should yield the normal values desired as basis of comparison for further work on low iodine goiter.

[3] Made by Purina Mills, St. Louis, Missouri.

EXPERIMENTAL PART

The animals were reared and maintained on the dog food referred to, and tap water, without other addition. They were kept on wire screens, in excellent sanitation. The sexes were kept separate and not permitted to reproduce. Killing was by chloroform, and the thyroids were immediately removed and placed in nickel boats approximately 50 × 7 mm. in size, and made especially for the purpose. To prevent loss of moisture, the boat was placed in a closed weighing bottle and weighed immediately. After all glands of any given group had been placed in the boat, it was placed in an oven at 100° until constant weight was reached, for determination of moisture. Iodine was determined on the entire contents of the boat by methods previously described (Remington, McClendon, von Kolnitz and Culp, '30).

In all ninety-four male and eighty-six female rats were used, ranging in age from 30 to 128 days, and in weight from 54 to 366 gm., with the exception of the last group of males and the last group of females shown in table 2, which were about 1 year of age.

RESULTS

a. Thyroid size

In table 2 are presented the results as actual fresh thyroid weight and thyroid weight per 100 gm. body weight. Each average value represents nine animals. The data were first tabulated for males and females separately and then recalculated for the sexes combined. As has been reported by others, we can find no significant sex difference during the period of rapid growth, or up to about 8 or 9 weeks of age, the thyroid gaining in weight at almost the same rate as the animal does. Thereafter, while the thyroid continues to grow, it does so less rapidly than the animal, so that when expressed in terms of 100 gm. body weight the thyroid values begin to decline after the animals reach about 150 to 180 gm. in weight. This decline is more rapid for the males than for the females

TABLE 2

Thyroid Weight in Relation to Body Weight

(Nine animals in each group)

SEXES COMBINED			MALES		
Weight	Thyroid Wt.	Per 100 g. Body Weight	Weight	Thyroid Wt.	Per 100 g. Body Weight
grams	mg.	mg.	grams	mg.	mg.
59 54- 62	7.0± 0.3 5.3- 8.3	11.9± 0.4 9.0-13.8	61 54- 74	7.0± 0.3 5.3- 8.7	11.6± 0.4 9.0-13.8
70 64- 76	6.8± 0.4 4.1- 9.1	9.7± 0.7 5.3-13.6	87 77- 96	9.0± 0.5 5.5-12.8	10.4± 0.6 6.4-14.5
83 77- 88	7.5± 0.4 5.5-11.6	9.0± 0.6 6.4-14.5	106 99-116	9.1± 0.3 6.9-10.9	8.6± 0.3 6.6-10.3
96 90-100	9.1± 0.5 5.4-12.8	9.6± 0.5 5.7-13.3	126 120-130	12.8± 0.4 10.6-14.9	10.0± 0.3 8.5-11.5
104 100-108	9.6± 0.5 6.9-13.3	9.2± 0.5 6.6-12.3	140 132-152	13.8± 0.6 8.7-17.6	10.0± 0.5 5.8-13.1
117 113-120	12.7± 0.6 9.3-17.2	10.9± 0.5 8.1-14.6	192 166-202	13.2± 0.3 11.9-15.9	6.9± 0.2 6.0- 6.3
124 120-128	12.2± 0.6 10.0-16.6	9.8± 0.5 8.0-12.7	218 208-222	16.9± 0.5 14.1-21.2	7.8± 0.2 6.4- 9.8
132 130-136	14.9± 0.6 10.8-20.0	11.4± 0.4 7.9-14.9	237 224-250	17.9± 0.5 14.6-20.9	7.6± 0.2 6.1- 8.9
141 138-144	14.1± 0.5 10.9-17.6	10.0± 0.4 7.8-11.5	268 252-280	17.6± 0.7 14.0-22.9	6.6± 0.3 5.5- 8.9
149 144-152	16.2± 0.7 8.7-19.0	10.9± 0.5 5.8-13.2	336 308-366	19.8± 0.8 14.0-25.8	5.9± 0.3 4.0- 7.4
156 154-158	17.5± 0.6 14.1-22.8	11.1± 0.4 10.0-14.8	455	20.6± 1.1	4.5± 0.3
165 160-168	15.4± 0.9 12.1-25.4	9.3± 0.6 7.4-15.5	FEMALES		
182 172-190	15.8± 0.6 11.9-20.2	8.8± 0.4 6.3-11.7	68 60- 76	6.7± 0.4 4.1- 9.1	10.0± 0.7 5.3-13.8
196 192-200	15.0± 0.7 12.0-21.6	7.6± 0.3 6.0-11.0	92 80-100	7.6± 0.4 5.4- 9.5	8.3± 0.3 5.7- 9.7
215 202-222	16.2± 0.6 12.4-21.2	7.5± 0.3 6.1- 9.8	115 108-122	12.8± 0.7 7.4-16.0	11.8± 0.6 6.9-14.6
234 222-246	17.9± 0.5 14.6-20.9	7.6± 0.2 6.1- 8.9	133 124-140	14.7± 0.7 10.0-20.0	11.0± 0.6 7.9-14.9
261 249-278	17.9± 0.7 14.0-22.9	6.8± 0.3 5.5- 8.9	147 142-152	16.4± 0.6 11.3-19.0	11.2± 0.4 7.8-13.2
292 280-308	21.9± 1.1 15.7-29.8	7.5± 0.3 5.6- 9.8	157 154-160	17.2± 0.7 12.1-22.8	10.9± 0.5 7.6-14.8
329 320-340	25.0± 1.4 16.1-34.7	7.6± 0.4 4.7-10.2	165 164-170	16.3± 0.9 12.1-25.4	9.9± 0.5 7.4-11.1
367 342-414	24.4± 1.7 14.0-41.3	7.6± 0.5 4.0-11.7	186 172-196	16.7± 0.6 13.0-21.6	8.9± 0.5 7.2-11.7
			309 249-340	26.8± 1.3 14.8-41.3	8.6± 0.3 5.9-11.7

because the males continue to gain in weight to a greater extent than the females. There is some evidence, more marked in females than in males, that there is a surge in thyroid growth at or about the time the animals reach sexual maturity. This was not anticipated, and so no provision was made for a sufficient concentration of animals in this particular area to firmly establish the observation.

Our values agree most nearly with those of McCarrison, but cannot be expressed by his curve for the reason that we find practically a constant value up to about 150 gm. body weight, whereas his equation yields a progressive decrease all the way. The values are lower also than those which we reported for rats reared on the Russell-Sherman diet. For the purpose of comparing with rats in which thyroid changes have been induced by diet or other means, 10 mg. per 100 gm. body weight can be used as a normal value (sexes combined) between 60 and 160 gm. weight, after which the normal gradually falls to about 7.5 mg. for 300-gm. rats.

b. Dry matter and iodine content

We believe this laboratory to have been the first to demonstrate that an edema of the thyroid is correlated with degree of iodine deficiency. In our earlier work ('33) we found that when potassium iodide was added to the diet in fully adequate amounts, the glands contained around 28% of dry matter, and that the dry matter content decreased with the iodine intake and iodine content, becoming in some cases as low as 20%. Since the animals used at that time were reared on the Russell-Sherman diet we were undoubtedly dealing with a condition of recovery, rather than with fully normal animals. In later experiments on the effect of iodine-bearing foods, and in which the young rats were produced on the dog food diet, values as high as 32% of dry matter have been obtained. In the present series the average value for sixteen groups comprising 131 animals is 33.3%, the same for females as for males (table 3). The range is from 30.5 to 36.9%, and there is no correlation with size of animals or iodine content of the glands.

As to iodine, several workers have reported that the thyroid of the rat contains less iodine than that of larger mammals (Sankaran, '30; Hercus and Roberts, '27; Cameron, '14). Orr and Leitch ('29) call attention to the possibility that a dietary iodine deficiency may be responsible. Sankaran, on the same glands used by McCarrison, to establish his normal thyroid curve, reported iodine contents of 0.007 to 0.042%, fresh basis. His average iodine was 0.0182 whereas the average value for our series of 118 animals on which dry matter and iodine determinations were made, is 0.066%, fresh basis; which figure corresponds with values found in the literature for cattle, hogs, rabbits and sheep, as well as for man (von Kolnitz and Remington, '33).

TABLE 3

Dry Matter and Iodine Content of Rat Thyroids

Males				Females			
Number of Animals	Average Weight	Dry Matter	Iodine Dry Basis	Number of Animals	Average Weight	Dry Matter	Iodine Dry Basis
	grams	per cent	per cent		grams	per cent	per cent
7	64	32.4	0.17	6	65	34.0	
18	117	34.3	0.18	8	93	35.4	0.20
11	122	31.3	0.19	8	125	31.9	0.20
9	211	32.6	0.20	7	131	32.9	0.19
10	225	33.3	0.18	7	153	32.2	0.22
7	246	35.6		7	157	30.9	0.20
8	277	36.9	0.20	7	173	35.7	0.22
5	326	30.5	0.21	6	317	33.8	0.23
Average		33.3	0.19			33.3	0.21

The wide variation in the iodine content of the glands of McCarrison's rats in itself indicates that deficiency must have existed in some of them, and if our value be taken as normal, they must all have been deficient. Why, then, are they not enlarged? We have previously shown (Remington and Levine, '36, plate 1) that noticeable enlargement does not occur unless the iodine content is as low as 0.05 to 0.06%, dry basis, or 0.013 to 0.015% fresh basis. Of the 107 glands analyzed by Sankaran, seventy-five contained 0.013% of iodine or more, and hence we would not expect them to be significantly enlarged. Based on our experience, however, microscopic examination of stained sections would reveal some irregular acini with partial softening or disappearance of colloid and possibly elongation of the basement cells.

SUMMARY

Measurements of the fresh weight, dry matter and iodine content of the thyroids of rats reared on an adequate and satisfactory diet containing 80 γ of iodine per 1000 Calories lead to the following conclusions:

1. During the period of rapid growth the thyroid weighs 10 mg. per 100 gm. of body weight for both sexes. Thereafter this value declines to about 7.5 mg. for fully grown rats.
2. The average dry matter content is 33.3%, and the average iodine content 0.20% on the dried gland, and there are no significant variations attributable to age or sex.

With the exception of those of McCarrison, the values for thyroid size are lower than found in the literature and the importance of an adequate amount of iodine in the diet is noted. The iodine content of the normal rat thyroid is of the same order of magnitude as that of domestic animals and man.

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A STUDY OF THE VASCULARITY OF THE PITUITARY BODY IN THE CAT

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THREE FIGURES

INTRODUCTION

In view of the importance placed on the endocrine system in the last decade and the very great physiological significance of the pituitary body or hypophysis, as well as with the idea of making more complete the knowledge of the quantitative vascular supply of the various regions of the brain, a study has been made of the capillary bed of the hypophysis of the cat, with particular reference to the relative vascularity of the different lobes. This work has been done during the past 3 years in the department of biology of the University of Toronto under the supervision of Dr. E. Horne Craigie, to whom the writer is greatly indebted for suggesting the problem and for assistance in carrying it through.

There have been many descriptions of the development of the pituitary body and its morphological structure, but it is generally agreed that, of the four lobes which may be recognized, the pars anterior, pars intermedia, and pars tuberalis, are derived from the ectoderm of Rathke's pouch, while the pars posterior is formed from the floor of the forebrain.

In 1908, Herring described three types of pituitary in mammals, and considered that of the cat to be the most primitive, i.e., the posterior lobe is hollow and in free communication with the third ventricle by the hollow stalk. The central cavity is lined with ependymal cells which send long fibers forward and up toward the brain. Tilney ('15) confirmed these observations as did de Beer ('23–'24). The latter published a diagram of a sagittal section of the pituitary of the cat which is reproduced in figure 1.

The extreme vascularity of the pars anterior of the pituitary has been noted by many observers, the first reference to the source of this supply being made by Duret (1873) when he described in the human species a small bilateral branch passing to the infundibular wall from the posterior communicating artery of the circle of Willis. In 1908, Herring stated that the anterior and posterior lobes of the pituitaries of the cat, dog, monkey, pig, ox, rabbit and man possessed independent blood supplies, that to the former coming down through the stalk, that to the latter entering it from behind, while the pars intermedia, investing the posterior lobe, was devoid of vessels. Dandy and Goetsch ('10–'11) investigated the blood supply of the pituitary in the dog, their results confirming and completing the conclusions of Herring with regard to the cat. They found that "the anterior lobe receives its blood supply from about 18 or 20 small arteries which converge toward the stalk from the various components of the circle of Willis. These vessels immediately break up in the stalk into numerous large sinusoidal channels in apposi-

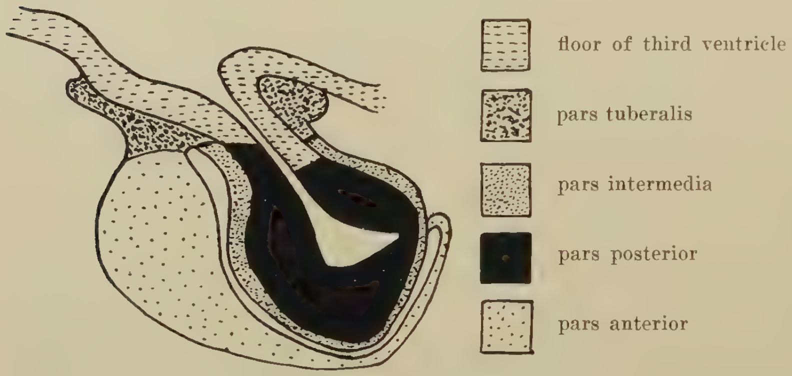

Fig. 1 Sagittal section through the pituitary complex of the adult cat. (After de Beer.)

tion with the cells. . . . The pars intermedia derives its supply from the vessels of the stalk, from the adjacent brain, and from the posterior lobe.'' By 'pars intermedia' they refer to those regions of the hypophysis which are now included in the terms pars intermedia and pars tuberalis. ''The posterior lobe receives its arterial supply from a small artery formed by the union of a symmetrical branch from each internal carotid.''

Brown ('24) published an article in which he states that the pars nervosa of the hypophysis of the rat has a rich supply of sinusoids forming a close network; that these are more numerous than in the pars anterior where the network is coarser and the vessels twice the diameter of those in the pars nervosa; that the pars intermedia is relatively non-vascular except at the periphery; and that the pars tuberalis has a rich blood supply. As will appear below, these observations were not completely confirmed by the writer in the case of the cat.

Nikolskaia ('29) found wide sinuses in the pars anterior of the human hypophysis while the supply to the pars posterior was similar to that of the surrounding brain tissue.

In 1930 Popa and Fielding described in the human subject, a portal circulation from the pituitary to the hypothalamic region. The hypophysio-portal veins drain blood from all the lobes of the pituitary and co-exist with the systemic veins which follow the course of the arteries. These two authors, however, do not agree with most writers on the the arterial supply to the various lobes. They describe a bi-lateral supply from the carotids, usually one artery on each side, to the pars anterior and pars intermedia, with smaller branches of this supply passing to the pars posterior, while the pars tuberalis receives branches from the circle of Willis.

Basir ('32) agreed with the findings of Dandy and Goetsch regarding the origins of the arterial supply although he also described the hypophysio-portal vein which carries blood from the pars tuberalis and pars nervosa into the tuber cinereum. The pars intermedia of Dandy and Goetsch is subdivided into pars intermedia and pars tuberalis, the former receiving its scanty supply from the vessels of the pars nervosa, the latter from the circle of Willis and the pars anterior.

This briefly outlines all the work hitherto published on the vascularity of the hypophysis, and reveals that so far no attempt has been made to express quantitatively the relative vascularities of the different lobes. It is the purpose of this paper to make such a comparison in the case of the cat, and to endeavor to interpret the results in the light of the most recent knowledge of their different physiological functions.

MATERIAL AND METHODS

The method employed in this investigation consisted in forcing a suitable injection mass into the blood vessels, replacing the blood by a substance which would harden and which could be measured on sectioning. The mass chosen was carmine gelatin prepared from commercial gelatin powder by the addition of carmine no. 40 (10 gm. carmine to one envelope of powder) which had been dissolved in distilled water with a few drops of strong ammonia. The mass thus obtained was treated with 20% acetic acid until dull scarlet in color and then filtered warm through very coarse filter paper. Berlin blue as used by Cobb and Talbott in their work was used once, but did not give satisfactory results.

The apparatus used for injecting the first ten cats was the same as that used by Dr. E. Horne Craigie in his work on vascularity, consisting of a hand syringe with a mercury U-tube as a pressure gauge. This method, beside being rather awkward, did not give very good results in the writer's hands, due, no doubt, to the difference in size between a cat and a rat and the necessity of giving so much more fluid, although it has the advantage of making the pressure increase gradually. During the past 2 years animals have been injected, compressed carbon dioxide being used to force in the mass, eight injections being successful. This method is much quicker and neater and can be controlled more easily.

Twenty-two cats in all, have been injected. However, on microscopic examination of the hypophysis only eleven were found to have the capillaries well filled, and one of these was from a female and was consequently not used in this study. As they were obtained from a dealer, no data can be given as to their age and breed. Every one was an adult male. The body lengths from tip of nose to base of tail of the animals finally used in the study are recorded in the following table. Each cat was killed by illuminating gas and as quickly as possible the thorax was opened, the abdominal arota ligated, and a cannula inserted through the left ventricle into the aortic arch and tied. The blood was washed out with warm isotonic saline followed by the warm injection mass. The pressure was increased gradually to the maximum value, also given in table 1. The amount of fluid given varied from 350 cc. to 500 cc., the injection being stopped when the membranes of the mouth and eyes were well-colored. This is not an absolute criterion for completeness of injection, however, since in some cases where the depth of coloration was very great, satisfactory results were not found in the sections. No. 11 was injected by H. Le Masurier of the department of anatomy who also used the CO_2 apparatus and who suggested its use for subsequent injections.

TABLE 1

	Cat 7	Cat 9	Cat 10	Cat 11	Cat 12	Cat 14	Cat 16	Cat 20	Cat 21	Cat 22
Body length,[1] inches	15	23	15.5	..	14.5	19.5	16.5	18.5	19.5	17.5
Maximum pressure reached, mm. Hg.	200	200	240	400	300	260	260	260	260	260

[1] Tip of nose to base of tail.

As soon as the injection was halted, the aorta was tied off, the neck of the animal ligated, and the head cut off and immersed in formalin for 12 hours. After this time, the brain and hypophysis were dissected out, the length, width and height were measured and the specimens again placed in formalin. After fixation for 12 hours more, the hypophysis was rinsed, dehydrated through graded alcohols to a clearing agent, and embedded in paraffin. Before embedding, each specimen was dropped into xylol, remeasured, and then left for a few minutes more in the paraffin. Sections were cut 20 μ thick (nos. 7, 9, 10, transversely, 11, 12, 14, 16, 20, 21, 22, sagittally) and counterstained with picric acid in absolute alcohol.

It is obvious that in the work outlined above there are many possible sources of experimental error. The difficulties of obtaining a complete injection are many. Aby (1899) discussed some of the problems arising from the substitution of a foreign mass for the blood, and Craigie ('20, '25) has dealt with others. The most serious question which arises is whether the figures obtained represent all the capillaries functioning at all times or whether there is a variation in the numbers of capillaries open from time to time. Cobb and Talbott ('27), experimenting with rabbits, found that the capillary bed changed on stimulation of neurones to the brain. This is in accord with conditions existing elsewhere in the body. Dandy ('13) who worked out the nerve supply to the pituitary in the dog, reported that from the carotid plexus of the sympathetic system numerous branches radiate to the stalk along the hypophyseal vessels to penetrate the anterior lobe. The supply to the posterior lobe is very scant while the pars intermedia receives its nerve supply from the stalk. Beattie ('32), who has been investigating the anatomy of the hypothalamic centers, has found that there is a rich nerve supply to the posterior lobe from the tuber nuclei as well as efferent fibers to the posterior lobe and pars intermedia from the supraoptic group of nuclei, and that the anterior lobe receives its innervation from the sympathetic via the carotid plexus (compare Dandy). The posterior hypothalamic nuclei send out fibers which descend to the upper thoracic levels from which preganglionic fibers pass to the anterior lobe, a circuitous route. Thus it is anatomically possible that the diameter of the vessels of the hypophysis may vary on suitable stimulation. The only way to insure complete injection is to use a pressure sufficiently high to force open all the capillaries. In one instance, cat no. 8, the posterior lobe count gave results that were reasonably close to those of well-injected specimens but the capillaries of the anterior lobe were obviously very incompletely filled. The pressure in this case did not exceed 160 mm. Hg. which is only 10 mm. Hg. above the normal blood pressure of the cat and considerably lower than the pressure attained when the injection was satisfactory. These facts give support to the above practice, and to the belief that the capillaries may expand or contract in localized areas independently. There is still another factor to be considered in the case of the posterior lobe. de Vries ('34), who injected cats with India ink, observed that during the flow of the experimental stream, also probably during life, the capillaries were much wider than in the mounted specimen. This may be due to a) active contraction of the Rouget cells, b) counter pressure of the brain tissue, or c) shrinkage due to fixation. According to him, in the brain, but not the anterior hypophysis and choroid plexus, which are not nervous tissue, the capillaries can be dilated by increasing the pressure, since the counter pressure of the brain tissue can be thus overcome. By varying his technique de Vries was able to produce laminar injection of the cortex by making use of the fact that tissue pressure is greater in the superficial layers than in the deeper ones. This effect is not marked with de Vries' normal injection pressure, and since in the present injections the pressure used was above the normal blood pressure for the cat, it would not introduce another source of error into the results.

Since the processes of fixation, dehydration, and impregnation caused a shrinkage in the tissue, it was necessary to apply a correction factor to the readings obtained. This factor was derived by measurement in three planes immediately after dissection and before final embedding, as described above. To the linear measurements thus made, the formula $P.E. = \pm 0.6745 \sqrt[2]{\frac{V^2}{n-1}}$ where V is the deviation of the individual percentage difference in length, due to shrinkage, from the average percentage difference (all reduced to a percentage of the original measurement when fresh), was applied —and the value of the coefficient found to be 10.1% ± 1.75. The figures obtained had therefore to be increased by $\frac{100}{90}$, and the measured volume multiplied by $(\frac{100}{90})^3$. It was necessary to work with percentages since it was impossible to make ten measurements of each dimension of the soft tissue, as such a practice would cause mutilation. It was felt that the number of measurements per hypophysis was insufficient to warrant application of an individual correction to each specimen. Also by using this method the measurements of fifteen specimens could be used to obtained a more reliable average.

The actual measurement was done on an area 200 μ × 200 μ in every other section of a series of twenty for the ten hypophyses used. Thus, the final result represents the length of the capillaries in a block of tissue 200 μ to a side. Using a Leitz-Edinger projection apparatus with Leitz ocular no. 0 and objective 6 lg, at a distance of 76 cm., giving a magnification of 420 diameters, the section was projected on a grid so constructed that one division represented 10 μ. As well as measuring the total length of the capillaries enclosed, the diameters of the individual vessels were measured and averaged for each hypophysis examined. Complete measurements were made in the anterior and posterior lobes and the results recorded in table 2.

It was not possible to identify the intermediate lobe in all ten specimens, and since its vascular supply was so scanty it was decided that a more representative figure would be obtained by counting the total lengths of the capillaries in all the pars intermedia in every twentieth section, and then reducing these values to fit those for pars anterior and pars posterior. These figures are recorded in table 2 as well.

Difficulty was also experienced in finding the pars tuberalis in all specimens. No. 10 was unfortunately cut at a peculiar angle making it impossible to locate the pars tuberalis and in no. 7 and no. 16 this region could not be identified clearly so that no counts were made in these specimens. In the other hypophyses, due to the small area of this lobe, only

TABLE 2

TOTAL OBSERVED LENGTHS IN μ OF THE CAPILLARIES IN $200^2 \times 200$ cμ TISSUE														AVERAGE DIAMETER	LENGTHS IN μ IN CUBE 100^3 cμ (FRESH)
	C 7	C 9	C 10	C 11	C 12	C 14	C 16	C 20	C 21	C 22	Average	P.E. of average, %	Corrected average in 200 cμ fresh tissue		
Pars anterior	7776	9409	8485	10591	6626	7954	10807	9021	10882	12029	9358	2.28	7486	7.7	936
Pars posterior	5622	7553	5494	7540	5315	7095	7415	6736	6885	10154	6981	5.58	5585	3.8	698
Pars intermedia	627	633		894	615		871	429	930	595	699	6.1	559	3.8	70
Pars tuberalis		9830		8383	6641	6884		6763	8335	8400	7891	1.2	6313	8.6	789

half the grid was counted. The values obtained were multiplied by 2 to give figures to correspond with those for the other lobes.

The validity of the figures recorded may well be questioned since it is very difficult to measure a twisting capillary and allow for the obliquity of its course and the thickness of the sections. However, since in each case a great number of vessels is involved the errors due to obliquity may cancel out. The value for the diameter is probably low, since there is a tendency for the gelatin mass to shrink away from the endothelium, and it is difficult to be sure that the longitudinal sections passed through a diameter and not just a tangential slice of the cylindrical vessel. This would be true in the case of the large capillaries of the anterior lobe especially.

That the results have statistical significance was shown by finding the P.E. of the average for each lobe and applying the formula, $\text{P.E.}_{x-y} = \sqrt[2]{\text{P.E.}_{x}^{2} + \text{P.E.}_{y}^{2}}$. In each case the difference was at least four times the root of the sum of the probable errors squared.

OBSERVATIONS

There could be no indecision in naming the anterior and posterior lobes in any of the sections studied. Although the picric acid counter-stain, an excellent background for the carmine of the capillaries, did not differentiate the tissue, nevertheless the granular nature of the cells of the anterior lobe could be made out and contrasted with the open mesh of glial tissue of the posterior lobe, surrounded and in some places invaded by a loose, open layer, varying in thickness, of finely granular cells, the pars intermedia. One specimen, no. 6, was stained with haematoxylin-eosin in order to assist in identifying the various lobes. The sagittal sections gave a clearer and more satisfactory picture of the arrangement of these lobes, and the connection of the lumen of the posterior lobe with the third ventricle by means of the stalk. Also, as the vessels in the anterior lobe were cut lengthwise a better idea of the loops could be obtained. The difference in degree of vascularity was obvious without measurement, the fine meshes of the posterior lobe contrasting sharply with the coarse network of the pars anterior and the practically non-vascular pars intermedia. The pars tuberalis, situated between pars intermedia and pars anterior and surrounding the stalk contained wide sinusoids into which the capillaries of the pars anterior empty.

From the table above it can be seen that the average diameter of the vessels in the anterior lobe is twice that of those in the posterior. This is in accordance with the findings of Brown in this connection, and the figures obtained agree very well with those of de Vries who gives 3 μ, and 5 to 9 μ. The values for the diameters of the vessels in the rat brain, recorded by Craigie ('20, '31, '33) as 2 to 4.5 μ show that the vessels of the posterior pituitary are similar in diameter to those of the rest of the brain, if the cerebral capillaries of the cat are of the same order of magnitude as those of the rat—a point yet to be demonstrated. The vessels of the intermediate lobe, branches of those of the posterior lobe, have the same diameter. The pars tuberalis has even wider sinusoids than the pars anterior.

The figures recorded indicate that in each case the length of the capillaries in the unit volume of tissue in the anterior lobe is greater than that in the posterior lobe, and the average relationship is 4:3. To obtain a more adequate representation of the degree of vascularity of these two lobes the diameters of the vessels must be considered. If the volume of capillaries in a cube 100 μ to a side is found, the ratio of vascular volume is found to be approximately 6:1. These results are in marked disagreement with the statement of Brown with reference to the rat: That the capillary sinusoids of the pars posterior are more numerous than those of the pars anterior. The accompanying photomicrographs of portions of a sagittal section through hypophysis no. 16 illustrate this difference in vascularity. Figure 3 also shows the practically non-vascular condition of the pars intermedia, which may be compared directly with the pars posterior since

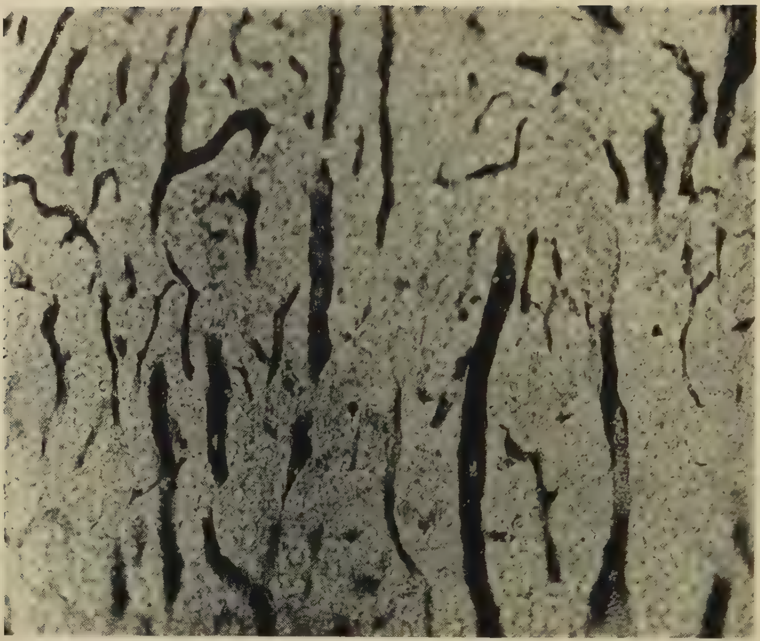

Fig. 2 Photomicrograph of a portion of a sagittal section through the anterior lobe of an injected hypophysis. × 250.

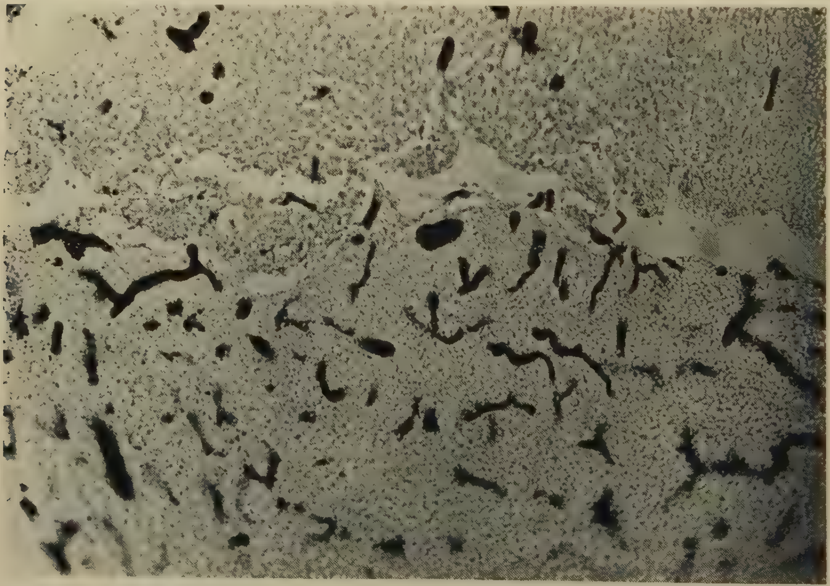

Fig. 3 Photomicrograph of a portion of a sagittal section through the posterior lobe and adjacent intermediate lobe of the same specimen represented in figure 2. The area with very few capillaries is the intermediate lobe. × 250.

their capillaries have the same diameter. The ratio of vascularity in this case approximates 0.1 : 1. However, a comparison of the vascularities of the pars tuberalis and pars posterior must be by volume of capillary supply, since the ratio thus obtained is 5 : 1, whereas if the ratio of supply were determined by length alone it would be more nearly 1 : 1.

To compare the values for the hypophysis of the cat with those obtained by Craigie for the various regions of the rat brain, the figures for the latter were multiplied by $(\frac{d}{2})^2$ to give the vascular volume (*d* was arbitrarily taken as 3.0). Compared on this basis, the anterior lobe has a richer supply than any region in the rat brain, the posterior lobe supply is similar to that of the nucleus motorius VII, and the intermediate lobe has a poorer supply than the least vascular center of the rat brain.

More significant in some respects than the volume of the vessels is the surface area of their walls, to which the volume of blood within them is exposed. Comparing the parts under consideration on this basis, we find that in a cube of fresh tissue of 100 μ edge the capillaries in the pars anterior of the hypophysis of the cat have a surface area of 22,631 sq.μ; those in the pars posterior an area of 8328 sq.μ; those in the pars intermedia an area of 835 sq.μ; and those in the pars tuberalis an area of 21,306 sq.μ (ratios of 27 : 10 : 1 : 25).

Calculating similar values from Craigie's ('24) figures for the brain of the rat aged 390 days, we obtain 3269 sq.μ for the capillaries in the medial longitudinal bundle (the poorest region which he studied) and 10,503 sq.μ for those in the dorsal cochlear nucleus (the richest of the parts he examined).

It might also be mentioned that no evidence was found to support the belief of Brander ('32) that a communication exists between the lower pole of the residual lumen and the cavernous sinus. Such a communication could also not be found by Rasmussen ('33) in the human hypophysis.

DISCUSSION

The observed difference in vascularity must have some significance in relation to the functional activity of the different lobes. Evans ('33) has summarized the present position of our knowledge of anterior pituitary function. We know that complete removal of the gland, though compatible with continuance of life, nevertheless results in abnormal conditions. We also know that cutting off the blood supply to the anterior lobe is equivalent to hypophysectomy. The conditions which arise are due to lack of the hormones produced by the anterior lobe secretion which is carried in the blood by the 'hypophysio-portal' circulation described by Popa and Fielding ('33) and Basir ('32), to the nuclei of the hypothalamus. From the anterior pituitary secretion four hormones have already been isolated. These have a marked effect on 1) growth; 2) development of the genital system; 3) secretion of milk; and 4) activity of the thyreoid gland. Alkaline extracts of anterior pituitary have a diabetogenic effect on normal dogs, although this hormone has not yet been completely separated from the others. The anterior pituitary is also linked functionally with the adrenal cortex, since on hypophysectomy the cortex atrophies and then may be stimulated to growth again by administration of anterior lobe extract. In pregnancy there is an increase in size of the anterior lobe correlated with the presence in the urine of the anterior-pituitary-like hormone. Wolfe and Cleveland ('33) found cyclic histological variation in the anterior hypophysis of the female albino rat. In the present study care was taken to use only male cats to avoid any complication from this effect. It would be interesting and worthwhile to investigate changes in vascularity, if any, corresponding with the histological variations described by Wolfe and Cleveland. Such work, however, could be carried out on the rat better than on the cat. From the foregoing statements it is obvious that the activity of the anterior lobe is very great, and its proper functioning is extremely necessary to the well-being of the individual. It is, therefore, not surprising that its vascular supply is so ample, since as Aby stated (1899) and all the work of Craigie ('20, '25, etc.) has borne out, vascular richness is related to functional activity.

The normal activity of the posterior lobe does not seem as essential to the individual as that of the anterior lobe, although its extract is of great value therapeutically. The active principle of this lobe is able to 1) raise the blood pressure, 2) contract smooth muscle, 3) cause hyperglycaemia, 4) expand cutaneous melanophores, and 5) diminish renal secretion. It is derived from the pars intermedia, the cells of which become basophilic when ripened (Rasmussen, '30) and under nervous stimulation from hypothalamic centers, transmitted by a rich supply of fibers described by Beattie ('32), are cast off by a kind of holocrine secretion, invade the pars nervosa and become transformed into 'hyaline' bodies, first mentioned by Herring ('08), which make their way through the loose neural spaces of the pars nervosa toward the infundibular cavity (Cushing, '33). This conception of posterior lobe secretion excludes the posterior lobe from among the endocrine glands, and removes the necessity of a rich network of blood vessels for the transference of the secretion. This is in accordance with the facts observed, that the posterior lobe has a much poorer vascular supply than the anterior lobe. However, when compared with the values found by Craigie in the rat brain, the vascularity of the posterior lobe is seen to approximate that of the nucleus motorius VII. This may be linked with the belief that stimulation of the posterior lobe induces activity in its epithelial investment, the intermediate lobe.

The very poor blood supply to the latter lobe does not correspond with its supposed role of elaborating the posterior lobe secretion. Some explanation of this condition may be advanced in the future but at present it is an unexpected discrepancy.

The pars tuberalis apparently serves as a relay station for the blood supply on the way to the hypothalamus. Why it is necessary to form a second capillary network here has also not as yet been explained.

CONCLUSIONS AND SUMMARY

The literature has revealed no quantitative measurement of the vascular supply of the different lobes of the pituitary. In the present study an attempt has been made to relieve this deficiency.

The total lengths and average diameters of the capillaries in a unit block of tissue in the four lobes have been measured anatomically. It is believed that the values recorded in the table give a fairly reliable comparison of the relative vascularity. The conclusions reached may be summarized as follows:

1. The anterior lobe of the hypophysis of the male cat is more richly supplied with blood than the posterior lobe, the ratio of their vascularity in terms of the volume of the vessels in a unit volume of tissue being 6:1. The average diameter of the vessels in the anterior lobe is twice that of those in the posterior lobe.

2. The posterior lobe, compared with centers in the brain of the rat, has a supply approximating that of the nucleus motorius VII. The diameter of the capillaries in the posterior lobe is of the same amplitude as that of the capillaries in the brain of the rat.

3. The pars intermedia is relatively very poor in capillaries, while the diameter of the vessels is the same as that of the posterior lobe vessels.

4. The pars tuberalis is richly supplied with blood sinusoids of a diameter even larger than that of those of the pars anterior.

5. The extreme vascularity of the anterior lobe corresponds with its very important functional activity. The posterior lobe, which does not seem so necessary physiologically, has a poorer supply. The intermediate lobe presents a paradox, being practically non-vascular and yet being said to elaborate the posterior lobe secretion. The function of the pars tuberalis has not been made clear as yet.

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BOOK REVIEW

THE PHYSIOLOGY AND PHARMACOLOGY OF THE PITUITARY BODY. by H. B. Van Dyke, professor of pharmacology, Peiping Union Medical College, Peiping, China. The University of Chicago Press, Chicago, 1936. 577 pp. $4.50.

In the foreword, Prof. A. J. Carlson aptly says that an attempt to describe and evaluate the scientific foundations of our knowledge of the hypophysis, is a herculean task, yet this monograph comes close to such an ideal.

There are fifty-five well chosen illustrations and over 3000 selected and complete references. The bibliography alone makes it almost invaluable to those especially interested in the pituitary body.

The initial chapter is devoted to anatomy, including the embryology, blood and nerve supply of the organ and its various parts. The author does not seem to be overly enthusiastic about the importance of the nerve supply. The weight of the different components of the hypophysis of man and nine species of animals are tabulated. Anatomical changes during pregnancy and following castration are reviewed.

Chapter II deals with the effect of hypophysectomy in various species. It is noteworthy that in the dog, at least, practically all the changes produced by the usual hypophysectomy are due to extirpation of the anterior lobe. The pars intermedia in this form is not considered to be of any physiological importance. The author also minimizes the importance of the neural lobe.

The related problems of the effects of hypothalamic lesions on metabolism of water, salts, carbohydrates, and fats are well stated. With reference to the discharge of neural lobe hormones into the cerebrospinal fluid the physiological evidence is considered far too equivocal to permit the acceptance of such a belief. In fact the evidence in favor of the neural lobe being an important gland is considered unsatisfactory and inconclusive.

In the chapter on the growth-promoting hormone, the timely statement is made that any generalization on the action of an extract must take into account differences in the response arising from variations in age, sex, race, diet, season, method and frequency of administration, ease of absorption and total dose. The hypophysis is not deemed of any importance in early embryonic growth, but in later embryonic life it possibly affects the rate of growth.

After birth the rate of growth, the ultimate body size and shape are dependent only in part upon the normal functioning of pars anterior. The genetic constitution of the individual is considered of equal or even greater importance. Yet the hypophysis is regarded as the most important of the ductless glands in regulating growth.

The effects of various types of anterior lobe substances on various animals are then detailed. The chapter closes with a discussion of the preparation, properties and potency of growth-promoting extracts of the anterior lobe.

Chapter IV takes up the gonadotropic effects of various derivatives of the anterior lobe. Apparently the human anterior lobe is one of the richest sources of gonadotropic hormone. Only two such hormones are considered to be reasonably well established—a) follicular stimulating and b) luteinizing.

Chapter V is devoted to a discussion of prolan—its origin, source, effects, preparation, detection and assay. Three pages are devoted to gonadotropic hormones in cases of malignant tumors of the genitalia.

The next chapter, on the effects of hypophyseal hormones on secretion of milk, is introduced by the statements that lactation cannot occur in hypophysectomized animals and that beyond this it is difficult to make generalizations. The assay and preparation of lactogenic extracts of the anterior lobe are then taken up. There is no evidence that the neural lobe is of any importance in lactation.

The interrelationship between the hypophysis and the thyroid is critically covered in the seventh chapter (30 pages). The relationship appears to involve only the anterior lobe.

Chapter VIII is concerned mostly with the pituitary-adrenal-pancreatic relations to metabolism of carbohydrates, fats and proteins. Then follows a short chapter on the pars intermedia and pars tuberalis and the hormonal regulation of chromatophores.

The next two chapters are devoted to the neural lobe and the oxytocic and the vasopressor principles derived therefrom. In spite of the yield of such active pharmacological products, the author concludes that close examination of the experimental data reveals that the evidence is unsatisfactory and still inconclusive with reference to this part of the hypophysis being a gland of internal secretion.

The final chapter takes up the effects of extracts of the neural lobe on metabolism of water, minerals, carbohydrates and fats.

In the appendix is a very useful list of scientific and commercial names of hormones and hormone preparations with a classification based on their source. The structural formulas of eleven hormones are also included.

Since the entire text is essentially an impartial summary of published data it is not possible in a review of this sort to adequately cover all the topics considered.

It should be highly welcomed by both clinical and laboratory investigators. The author is to be congratulated on having brought together so much of the worthwhile information on this master gland.

The book is well bound and surprisingly free from typographical errors.

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University of Minnesota

NEW BOOKS AND PUBLICATIONS

AN INTRODUCTION TO COMPARATIVE BIOCHEMISTRY, by Ernest Baldwin, with a foreword by Frederick Gowland Hopkins. 1937. Size, 5 × 7½ inches. Pages xviii + 112. 11 illustrations, 9 charts. Index. Bibliography. Cloth. Price, $1.50. Cambridge: at The University Press. The Macmillan Company, New York.

A survey of the aims and possibilities of biochemistry is contained in this introduction. The student entering on the study of the chemical nature and function of animals should acquire some comprehension of its scope before he becomes immersed in details. Assuming that life began in the sea, the first living creatures were perfectly adapted to surroundings; and chemical conditions inside the organism were practically identical with those outside it. In attempts to colonize fresh water and dry land, only those forms succeeded which could preserve their internal environment in the midst of external changes. The far-reaching adaptations are traced in the evolution of the ''self-buffering, automatic thermostat which is man.''

TRANSACTIONS OF THE WESTERN SURGICAL ASSOCIATION, Forty-fifth Annual Meeting, Rochester, Minnesota, December 6 and 7, 1935. 1936. Size, 6 × 9 inches. 610 pages. Lists of officers and members. Report of proceedings. Index. Illustrated. Cloth. The Bruce Publishing Company, St. Paul.

The twenty-five papers and discussions presented during the forty-fifth annual meeting of the association cover a wide range of subjects. Historical aspects are represented by the President's address entitled ''The influence of the West on American Surgery,'' by an article on Andreas Vesalius, and an outline of the growth of specialization in the medical profession. The rise of specialization has been traced from the days B.C. when the only medical man was the family physician, until today when nearly 150 specialties are listed. The discussion following the paper reflects general professional opinion on the subject. As one member expressed it ''a real specialist is a rara avis—he is made, not born, and the way is long and arduous.'' Other articles describe problems, advantages and refinements of modern surgical practise, operative technique and post-operative care.

HANDBUCH DER ANATOMIE DES KINDES, edited by Karl Peter, Georg Wetzel and Friedrich Heiderich. Erster Band, 4 Lieferung. Systematische Anatomie der Organe I. Atmungsapparat (Apparatus respiratorius) Der Kehlkopf des Neugeborenen, by K. Peter. Die Luftröhre und die Lungen des Kindes, by G. Wetzel. 1936. Size, 6¾ × 10½ inches. 104 pages. 54 illustrations. Lists of references. Price, RM 24.00. J. F. Bergmann, München.

This section describes the embryology and anatomy of larynx, trachea and lungs. The volume has been issued in sections which were sold separately. It will be completed with the fifth lieferung which will be ready in the spring and from now on the volume will be sold complete instead of in separate parts.

WHY QUIT OUR OWN, by George N. Peek with Samuel Crowther. 1936. Size, 5½ × 8½ inches. 353 pages. Cardboard. Price, $0.50. D. Van Nostrand Company, Inc., New York.

One of the authors of this political treatise is a former administrator of the agricultural adjustment act and special advisor to the president on foreign trade, who retired when his proposed policies on farm and foreign affairs were rejected. The first chapter describes his experiences in politics, with excerpts from his diary. The main body of the text tells what is wrong with the United States and what should be done about it, with quantities of statistics to prove it.

A CASE OF SIMULTANEOUS OCCURRENCE OF A NUMBER OF VARIATIONS OF THE VISCERAL BRANCHES OF THE ABDOMINAL AORTA

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ONE FIGURE

In the course of dissection work with students, I have come upon a case of a combination of variations of the visceral branches of the abdominal aorta, which is of interest since it has not been so far described in the literature available to me, and also because of its importance from a surgical point of view.

DESCRIPTION

On the body of a Russian woman, 39 years old, the following was found: From the abdominal aorta, at the level of the twelfth thoracic vertebra, arises a short trunk, 4 mm. long, as a result of the fusion of art. coeliaca and art. mesenterica superior, in the form of truncus coeliaco-mesentericus (Adachi) or simply arteria coeliaco-mesenterica (Tichomiroff, Hyrtl). This trunk, at a distance of 4 mm. separates into its two component arteries: art. coeliaca and art. mesenterica superior. In its turn, art. coeliaca, having a length of 4.8 mm., is directed forward and at different levels gives off not three, as is usual, but only two branches: a. gastrica sinistra and a. lienalis, forming, according to Adachi, truncus gastrolienalis. Art. hepatica, third branch of the usual coeliac artery, branches off from the art. mesenterica superior and lies back of the portal vein, forming the so-called 'typus hepato-mesentericus' Adachi. The second, and very rare, variation, is the unusual branching off of art. gastro-duodenalis from the splenic artery instead of its typical origin from the hepatic artery. From the proximal part of the above described truncus gastro-lienalis, at a distance of 1 cm. from its base, originates the art. gastrica sinistra, which proceeds

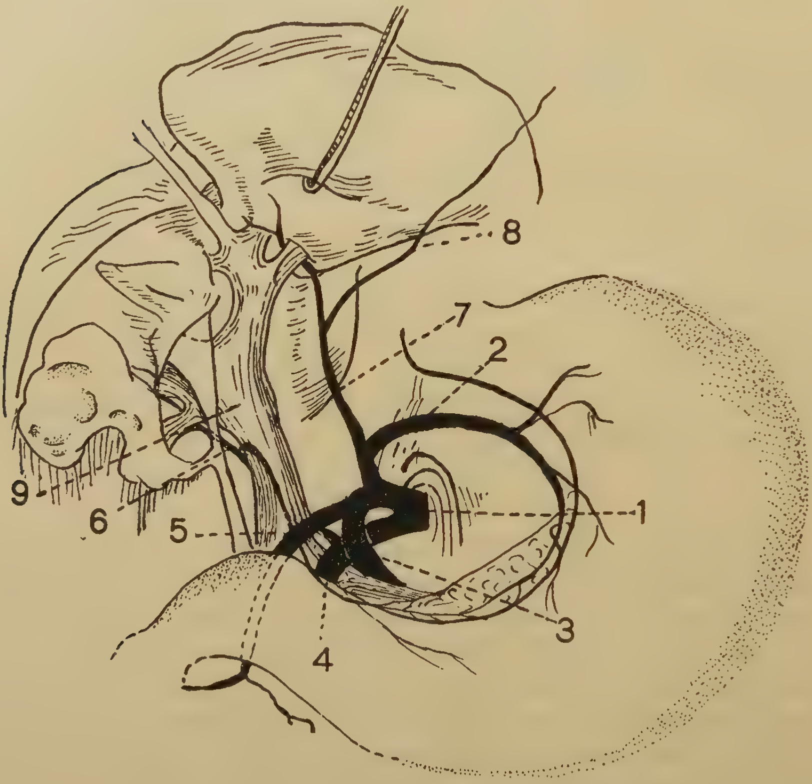

Fig. 1 Variations of the abdominal aorta. 1, truncus 'gastro-lieno-mesentericus' is composed of: 2, a. gastrica; 3, a. lienalis; 4, a. mesenterica superior. 5, a. gastrico-duodenalis originates at a. lienalis (a very rare case). 6, a. hepatica accessoria dextra, forming 'Typus hepato-mesentericus' Adachi. 7, a. hepatic accessoria sinistra (branches off from a. gastrica sinistra). 8, a. phrenica inferior dextra (branches off from a. hepatica accessoria sinistra). 9, v. portae.

toward the small curvature of the stomach; while from the distal end originates a second branch, art. lienalis, forming, as it were, a continuation of the trunk itself. Both of these arteries, proceeding toward the respective organs, on the way give a series of unusual branches, as follows: from the a. gastrica sinistra branches off an additional left hepatic artery —a. hepatica accessoria sinistra; this, before reaching the left lobe of the liver also gives off an additional branch toward the diaphragm, replacing the absent a. phrenica inferior, which usually arises from the coeliac artery or from the aorta. A. lienalis, which forms the second branch of the truncus gastro-lienalis has its typical direction and usual diameter, and, in addition to the usual branches, gives off also from its immediate origin a. gastro-duodenalis, which, as a rule, originates at the hepatic artery.

DISCUSSION

According to anatomical texts, as well as the periodical literature, the first unpaired visceral branch is termed 'arteria coeliaca.' However, Adachi calls it 'truncus' coeliacus, and explains that the term 'coeliaca' is applicable only in case the above-mentioned trunk gives off three typical arteries: a. hepatica, a. gastrica sinistra and a. lienalis, i.e., there is formed the truncus hepato-gastro-lienalis or tripus Halleri; even if from a. coeliaca, in addition to the three basic branches above referred to, there are additional branches: a. a. hepaticae accessoriae, a. gastrica sinistra accessoria, a. phrenica inferior, but not a. mesenterica superior, the coeliac artery still preserves its name. However, in cases in which only two and not three branches exist, it loses its designation as 'coeliac' and becomes truncus gastro-lienalis or truncus hepato-lienalis. Rauber, on the other hand, preserves the designation 'coeliac' also in case of the existence of only two branches, as well as in cases of a supernormal number of branches.

In our case, we have a combination of two variations: The presence of the truncus gastro-lienalis, which forms a part of the absent typical form of arteria coeliaca, and, second, the fusion of this truncus with the a. mesenterica superior, which forms the first combined variation, described in this form neither by Adachi, nor elsewhere in the literature, and to which we have given the name 'truncus gastro-lieno-mesentericus.' The second very rare variation—origin of art. gastro-duodenalis not from the hepatic but from a. lienalis, has been described only by Adachi, in one case out of 252 Janpanese, forming 0.4%. This variation has not been described by other authors. There are indications in Rauber and Tichomiroff concerning irregular origin of a. gastro-duodenalis, not from the splenic artery as in our case, but from the superior mesenteric artery. Our case may thus be considered as the second of its kind in the literature.

As far as the other variations observed in our case are concerned, the origin of the hepatic artery at a. mesenterica superior and its position dorsal to the portal vein is a relatively frequent variation—according to Rio Branco in 4% of cases, according to Sousloff, 4.3%, as contrasted with the very rare cases of a. hepatica being situated ventrally from v. portae; this latter situation is represented by only two cases in the literature: One described by Adachi and the other by Lyssenkow.

SUMMARY

On the body of a Russian woman, 39 years old, the following was observed: From the abdominal aorta, at the level of the twelfth thoracic vertebra, arises a short trunk 4 mm. long which gives off a number of branches not usually found. Among these the following are rare: 1) Fusion of a. mesenterica superior with the incomplete trunk of the coeliac artery, termed truncus gastro-lienalis, by Adachi, forming altogether truncus gastro-lieno-mesentericus, not so far described in the literature. 2) Irregular, very rare (0.4% of cases) origin of a. gastro-duodenalis at the a. lienalis, instead of its usual origin at the a. hepatica (in our case missing), as a third branch of a. coeliaca. 3) Irregular origin of a. hepatica from a. mesenterica superior, instead of its origin from a. coeliaca in the form of typus hepato-mesentericus (for the right lobe of the liver). 4) Origin of the left additional hepatic artery, a. hepatica accessoria sinistra from a. gastrica sinistra (for the

left lobe of the liver). 5) Irregular origin of a. phrenica inferior dextra from a. hepatica accessoria sinistra.

As far as the nature of the origin of these variations is concerned, it is hardly possible to regard them as atavistic, since they have a distinctly progressive character, as increasing the branching of the various arteries of the abdominal cavity. Since many anastomoses are present in the branches of the abdominal aorta in the embryonic stage (Tandler), it is possible to explain our variations as remnants of such anastomoses.

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A METHOD FOR VISUALIZING DIFFERENT ORGANS IN THE NORMAL UNANESTHETIZED ANIMAL

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ONE PLATE

For a number of years certain radiologists (Yater and Otell, '33) have injected intravenously a colloidal suspension of thorium dioxide (Thorotrast) to visualize the liver, spleen or parts of the circulatory system in clinical subjects. The advantages of this procedure are that Thorotrast is miscible with blood, and that it is taken out of the blood stream by the reticulo-endothelial system of the liver and spleen which in turn remain opaque to x-rays. Some, on the other hand, object to the use of the material because of its slight radioactivity which has caused tumorous growths in rats (Selbie, '36). To our knowledge no such effects have as yet been reported in the human.

Since the advantages of this technique seemingly outweighed the disadvantages, an attempt was made to use this material to visualize the organs in experimental animals. Instead of injecting the Thorotrast into the blood stream from which it soon disappears to be stored in the reticulo-endothelial cells, the technique was revised in this way: It was injected directly into the walls of organs, where it remained permanently. Since one of us (FRS) was interested particularly in the physiology of the colon, experiments were first done upon the walls of the colon. This was done by sterile operation, exposing the colon and injecting the Thorotrast just underneath the serous membrane in various places along its entire length.

For the first 2 weeks after the injection, the x-ray pictures showed the material distributed more or less in patches, but after 3 or 4 weeks it appeared to be quite uniformly distributed and presented a very satisfactory outline of the colon (plate 1). The distribution, no doubt, was related to the peristaltic movements and return of muscle tone in the colon.

As to whether any injury to the tissue resulted from the presence of the material, little can be said at the present time other than that we now have four apparently healthy cats with their colons injected with the material, one injected over 16 months ago and the remaining three more than 7 months. Judging from the health, appetite and stools of the cats it is believed that the colons are in a normal physiological condition for experimental study; with this preparation one can study the movements in the empty as well as the distended colon. Since, however, tumorous growths have been found in rats and mice injected subcutaneously with Thorotrast, it is essential that these cats be thoroughly examined for pathological changes in case of death.

More recently similar experiments were performed upon the gall bladder, urinary bladder and the stomach of the cat, as well as the crop, gizzard, and uterus of the fowl, with results which promise to be just as satisfactory as those obtained on the colon.

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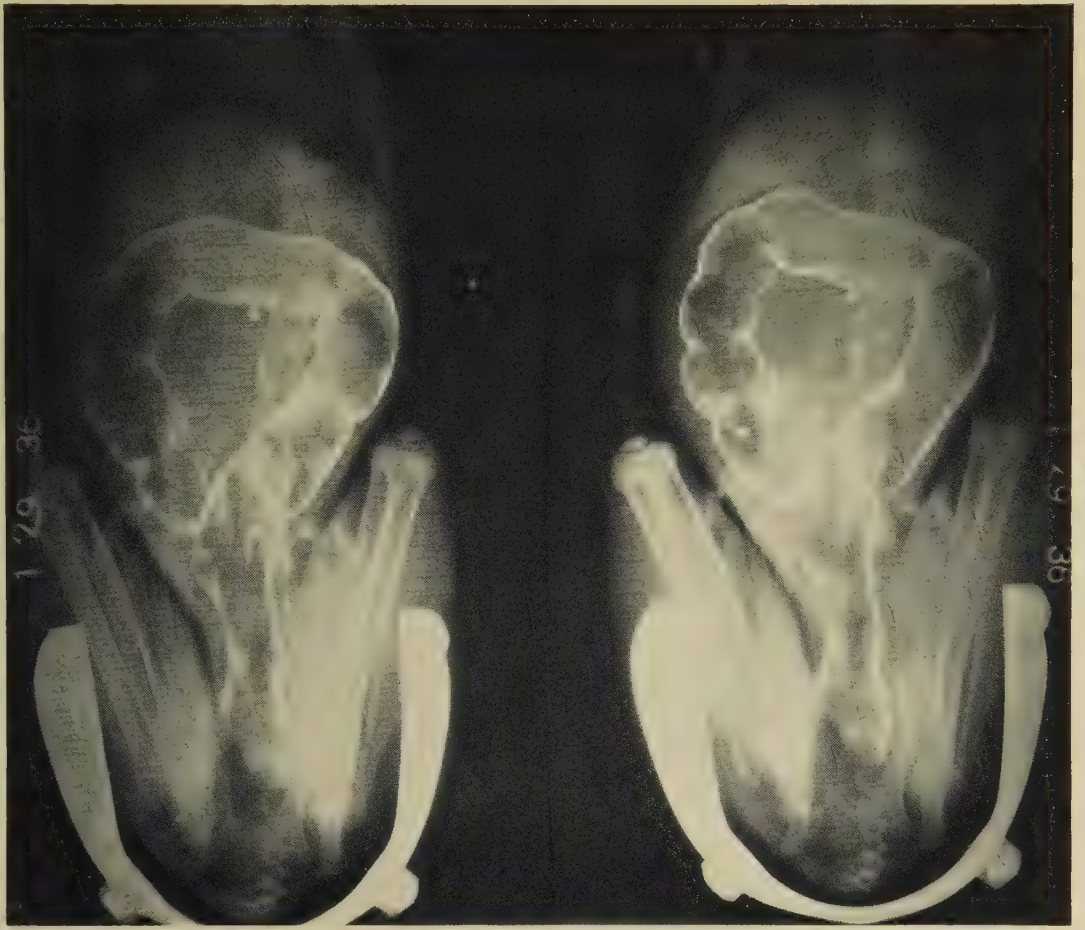

The x-ray plate on the right was taken 2 minutes after that on the left. Note the difference in the position of the peristaltic contractions. (Pictures taken from cat still alive 16 months after injection.)

METAMORPHOSIS OF THE PUBIC SYMPHYSIS

III. HISTOLOGICAL CHANGES IN THE SYMPHYSIS OF THE PREGNANT GUINEA PIG

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ONE PLATE (FIVE FIGURES)

The phenomenon of the relaxation of the pelvic joints, and the separation of the pubic bones at the symphysis, with formation of a symphyseal ligament in the pregnant guinea pig, was observed and described by various authors considerably in advance of the actual experimental demonstration of the causes of this metamorphosis. The truth of Todd's ('23) statement that "there is probably no single fact in comparative anatomy which has been more often rediscovered than the extraordinary separation at the symphysis of the guinea pig in late pregnancy and parturition" is obvious to those who are familiar with the literature on this subject. It is of historical interest, however, to note that Bland-Sutton ('11), who erroneously believed that he was the first to describe the pubic ligament of the parturient female guinea pig, states that "a microscopic examination of the symphyseal ligament shows nothing to explain its extensibility."

In a recent investigation (Ruth, '36) it has been shown that the pubic symphysis of the female guinea pig develops in the same manner as that of the male, with an uninterrupted plate of hyaline cartilage between the osseous elements, until the latter part of the gestation period. At that time a sexual dimorphism appears, through a cataplasia of the interosseous plate of cartilage. By this process a fissure appears, and finally causes the separation of the osseous elements, with a concomitant elaboration of collagenous fibers in the form of a connecting ligament. This metamorphosis makes possible the spectacular separation of the osseous symphyseal elements (pubic and ischial rami) during parturition, and is accompanied by a hyperplasia of the symphyseal ligament itself, which allows "the delivery of a fetus having an average head diameter of 20 mm. through a pelvic canal with an average diameter of 11 mm." (LeGallois, 1812). The present investigation is concerned with the histological changes observed at the symphysis of pregnant females during gestation, parturition and to the fourth week postpartum.

MATERIAL AND METHODS

Thirty female guinea pigs (half of which were nulliparous) were used in this investigation. Daily examinations were made to detect the occurrence of oestrus in each individual. Each female was placed in a breeding cage with a male as soon as the vagina opened. When examination revealed closure of the vagina, the female was placed back in the cage with the other females. After an individual had been in the breeding cage, and then failed to come into oestrus at the expected time, she was placed in a cage for pregnant females and considered pregnant unless subsequent examinations proved otherwise. The daily examination was continued until fetuses could be palpated and the diagnosis of pregnancy definitely confirmed. Each female was marked and separate records were kept for each one. When the diagnosis of pregnancy was confirmed by the palpation of fetuses, the number of days of gestation were counted from the date of closure of the vagina. Pregnant animals were sacrificed at weekly intervals from the eighteenth day of gestation to and including the day of parturition. Subsequently, animals were killed every third day postpartum for 2 weeks, and again at the end of the fourth week. The symphyses were fixed in Zenker's, and embedded in paraffin. Sections were stained with hematoxylin and eosin, and with Mallory's triple connective tissue stain.

During the first 5 weeks of pregnancy little change is to be noted at the symphysis. The connective tissue forming the ligament is arranged in bundles of collagenous fibers which run antero-posteriorly, in the direction of the long axis of the symphysis. These gradually shift into a transverse direction at the anterior and posterior extremity of the joint, to become continuous with the anterior and posterior pubic ligaments. The ligament is practically avascular, and during this period nothing remarkable is to be noted—the joint appearing much like that of a non-pregnant animal.

After the fifth week of gestation a gradual invasion of the symphyseal ligament by capillaries can be noted. With the appearance of capillaries there is a noticeable widening of the ligament. One or two small islets of hyaline cartilage, unassociated with other cartilage or bone of the joint, were found in the ligaments of a few animals at this time. The increase in vascularity becomes more apparent as gestation progresses toward term, and the vascular channels become greatly enlarged. This enlargement of the capillaries is especially noticeable during the last two weeks of gestation.

The histological picture at the symphysis varies somewhat in different females, according to age and to changes brought about by previous pregnancies. At any given stage of pregnancy the symphyseal ligament of the multiparous female is wider than that of the female that has not borne young before.

The extent to which the pubic and ischial bones are covered with cartilage along the symphyseal face varies with the age of the animal. In the older females the cartilage is almost wholly absent, and the fibrous tissue of the ligament is attached directly to the periosteum.

By the eighth week the vascular channels of the symphyseal ligament have increased noticeably in size and number. Actual resorption of the symphyseal faces of the osseous elements has proceeded to the stage where the medullary cavities of the bones concerned have actually been opened and invaded by the fibrous tissue of the ligament. The numerous vascular channels of the ligament anastomose with, or in some cases arise from the vascular channels of the bone (fig. 2). Indeed it seems that the opening of the vascular channels of the bone is the more important process through which the spectacular vascularization of the ligament during late pregnancy and parturition is effected. Bone resorption in this case contributes only secondarily to the widening of the birth canal, as the width of the bone destroyed is not at all remarkable.

The vascularization of the ligament and its resultant hyperplasia apparently is concomitant with the appearance of osteoclasts along the symphyseal faces of the pubis and the ischium, between the bone and the ligament (fig. 1). However, the number of osteoclasts present is not extraordinary, and would seem wholly inadequate to cause the relatively extensive resorption of bone by any local osteolytic action at the points at which they are found. Rather it would seem that the resorption of the bone is the stimulus which results in the formation of these giant multinucleated cells. These in turn probably assume a phagocytic activity for the removal of any resulting osseous debris.

The osseous elements of the symphysis by this time show unmistakable evidence that considerable bone resorption is occurring. The actual opening up of the marrow cavity in many places (as mentioned above) is perhaps the most apparent indication that bone is being removed. In addition to these actual openings in the symphyseal plates of the ischium and pubis, the whole plate throughout is noticeably thinned, and in many places has become so thin that only an extremely narrow wall of bone intervenes between the marrow cavity and the ligament. As gestation draws near to term, even these thin walls of bone are resorbed, and extensive areas of the medullary space are opened up. The myeloid elements disappear, and the space is filled with fibrous tissue that is continuous with the symphyseal ligament. In such areas only the lateral plate of the ischium and pubis remains (fig. 4). The dissolving away of the bone is shown by the appearance of canals or cavities in the symphyseal plates of the bones concerned. These become more numerous and extensive until the walls of bone between them become thin and finally are removed, permitting adjacent spaces to coalesce, and resulting in a trabeculated appearance of the plates. In some areas there is actual necrosis of the bone, and resorption occurs by the phenomenon of 'creeping replacement' (Ham, '32). By this means new bone forms in minute and discrete islands around osteocytes bordering the necrosed area, thereby exerting pressure on the area of dead bone. Humoral agents, possibly elaborated by the osteocytes themselves, cause the removal of the dead bone, while the chemical action is accelerated by the pressure exerted by the encroaching islets of new bone (fig. 5).

The greatest change at the ischio-pubic symphysis of the pregnant female occurs during the ninth and last week of gestation. This is seen in the marked hyperplasia of the symphyseal ligament. Up to this time the lengthening of the ligament has been relatively gradual, but now vascularization and proliferation of the connective tissue elements increase at a tremendous rate, the latter process being dependent upon the former. The fiber bundles, which run parallel to the antero-posterior axis of the joint during early pregnancy, tend to change direction as the ligament lengthens through the proliferation of the fibrous tissue. At the onset of this shift in direction of the fiber bundles they tend to bend away from their original position toward a more transverse direction, especially those fibers near the middle of the ligament. The fibers immediately adjacent to the bone, on the other hand, retain their antero-posterior direction throughout pregnancy. As the fiber bundles toward the middle of the ligament progressively change direction they appear in the histological sections to run in many different directions until the last week of gestation, when (with the great acceleration of proliferation of fibrous tissue) these middle bundles assume a definite direction at right angles to those fibers immediately adjacent to the symphyseal faces of the bones. The rapidly proliferating fibroblasts have the typical appearance of such cells in multiplication—the young cytoplasm taking a light basic stain, and the nucleus being relatively large, distinctly vesicular, and varying from spherical to oval in shape.

The extraordinary vascularization of the symphyseal ligament is developed to the highest degree at parturition (fig. 3). Arterioles and capillaries permeate the ligament in all directions, but show a general tendency to run parallel with the long axis. The vessels vary in size from capillaries 7 μ in diameter to the larger arterioles which average 0.1 mm. in diameter. In many places there is considerable hemorrhage into the ligament—more or less extensive aggregations of erythrocytes being found free among the bundles of fibers. In other areas relatively great extravasations of blood are found in large sinusoidal spaces measuring as much as 1 mm. in diameter and 1.5 mm. to 2.0 mm. in length. Some of these blood-filled sinuses have a definite endothelial lining, although it may not be continuous, while others apparently lack a definite endothelium. In several of the microscopic sections arterioles and capillaries can be seen actually emptying into these blood spaces.

After parturition the regressive changes in the symphyseal ligament are marked and rapid. By the third day postpartum the ligament has shortened approximately by half. The numerous blood channels and spaces have been reduced in size and number, and most of those that remain are empty, except those vessels immediately adjacent to the periosteum. Already the fiber bundles show a tendency to move into the antero-posterior direction characteristic of the non-pregnant or early pregnant animal, and the beginning of the repair of the symphyseal faces of the pubis and ischium is apparent.

Bone repair is accelerated to such an extent that the symphyseal plates of the ischium and pubis have been regenerated by the end of the second week. The fibrous tissue has withdrawn from the medullary space, and the new cavity contains the usual myeloid elements. The method by which the bone is regenerated suggests the development of membrane bone, inasmuch as there is no new formation of cartilage. The abundant active young fibroblasts are metamorphosed into osteogenic cells along the line of bone formation, and osteoid tissue is deposited by their activity. At this time the ischio-pubic joint has returned to the condition observed in the non-pregnant multiparous female. The bundles of fibers again are passing in an antero-posterior direction, and the ligament is practically avascular. The cells (fibroblasts) have returned to a resting condition with the elongated form of cell and the dense elongated nucleus that is typical of dense fibrous connective tissue. The histological picture as described 2 weeks postpartum remains unchanged at the fourth week, and it seems reasonable to assume that the symphyseal response to pregnancy has been fully repaired.

DISCUSSION

The phenomenal hyperplasia and accompanying vascularization of the symphyseal ligament of the guinea pig during the last week of gestation may be readily compared with those changes accompanying connective tissue repair. Indeed, the whole course of the progressive and regressive metamorphosis in the ligament during the last week of pregnancy and for 2 weeks following parturition is almost identical histologically with the phenomenon of repair of connective tissue. A spectacular invasion of the fiber bundles by arterioles and capillaries, accompanied by an extremely active proliferation of fibrous tissue elements is characteristic of both cases. The final withdrawal of vascular channels and the consolidation of the cicatrix in the case of connective tissue repair is likewise paralleled by the similar vascular withdrawal and the concomitant atrophy of the connective tissue which results in a shortening of the symphyseal ligament. Concerning cicatrization Karsner ('26) states that "the purpose of the rich vascularization in organization is to provide nutrition for the rapidly multiplying cells. When multiplication stops, the need for capillaries no longer exists, and their atrophy may be a manifestation of adaptation to the changed conditions . . . . It (the scar) gradually becomes paler . . . . at the same time decreasing in size and contracting until it is likely to be smaller than the original injured area. With the shrinkage comes increased density.''

Kawata ('24) reports an investigation of the histological changes at the ischio-pubic symphysis of the pregnant guinea pig, in which he states that ''the most interesting histological finding is the remarkable proliferation of the osteoclasts along the symphyseal surface of the pubis and ischium during pregnancy, especially near the time of parturition. Some parts of the bones are even perforated to the marrow.'' He explains the widening of the space between the osseous elements of the symphysis as being due to destruction of the margins of the bones (inferring that this destruction is due to the activity of the osteoclasts), mucous degeneration of the symphyseal connective tissue, and fibrosis of ''both fibrocartilage and hyaline cartilage over the bones.''

As has been noted on page 412, the author was unable to find osteoclasts in any unusual number in the specimens studied, nor was there evidence of mucous degeneration. Fibrosis of the cartilage covering the symphyseal surfaces of the bones (in a female young enough to have such cartilage) would contribute relatively little to the widening of the ligament. On the other hand, it has been demonstrated that the outstanding phenomenon in the separation of the osseous elements of the symphysis at parturition is the pronounced hyperplasia of the fibrous tissue through a tremendous proliferation of the connective tissue cells of the ligament.

The ligament itself is made up of dense bundles of collagenous fibers, with a few elastic fibers. It is not yet possible to explain fully the mechanism controlling the lengthening of the symphyseal ligament during gestation. It seems probable, however, that the stimulus resulting in the formation of the rapidly multiplying fibroblasts by an active proliferation of the original connective tissue, is of hormonal origin (Hisaw, '29).

The actual mechanism of the bone resorption is not known. No one of the several theories offered to explain why and how bone is resorbed adequately covers all of the phenomena observed in the various normal and pathological conditions where resorption occurs. Resorption by the specific osteolytic activity of the multinucleated giant cells, known as osteoclasts, is a concept that is no longer universally accepted. Two outstanding objections to this theory might be mentioned: 1) When these cells are present in an area in which resorption is occurring, they are seldom in sufficient concentration to impress the observer that it is their osteolytic action that is dissolving away the bone; and 2) in those pathological cases wherein resorption progresses in the complete absence of osteoclasts the postulation of a specific localized osteoclastic activity would be untenable.

In the present instance the extensive bone resorption that is seen in the ischio-pubic symphysis cannot be accounted for by the local osteoclastic activity of the relatively few osteoclasts that are found in the histological sections. No osteoclasts at all are found in intimate relation to the areas of necrotic bone. For this reason the author adopts the view that resorption is brought about by 'creeping replacement' (as already explained) in the areas where bone necrosis is observed. Resorption in other areas is under humoral control, with the osteoclasts acting as phagocytes for the removal of osteoid debris after the inorganic salts are dissolved away.

SUMMARY

The notable histological changes at the ischio-pubic symphysis of the pregnant female guinea pig appear after the fifth week of pregnancy. These changes are slow at first, and consist of a progressive vascularization of the symphyseal ligament, accompanied by a proliferation of the connective tissue cells. The resultant lengthening of the ligament is gradual and at first almost imperceptible. Bone resorption is noted toward the end of gestation but probably begins much earlier. It is so gradual that it would be difficult to say when it first begins, but the end result is the complete resorption of relatively extensive areas of the medial tables of the pubis and ischium, with the resultant opening up of the respective medullary spaces.

Bone resorption, vascularization of the symphyseal ligament, and proliferation of the connective tissue cells show a great increase in activity after the eighth week of gestation, and move rapidly toward the climax of these processes at parturition. At parturition the ligament is found to be lengthened greatly through a hyperplasia of the connective tissue elements. Vascular channels appear in large numbers, and many of them open into blood spaces where considerable amounts of blood have collected. The medullary spaces opened by bone resorption are invaded by fibrous tissue, and all myeloid elements are lost.

After parturition regressive changes comparable to cicatrization are seen in the symphyseal ligament, while bone regeneration with the reappearance of myeloid elements in the medullary spaces is relatively rapid. Complete repair is observed as early as the end of the second week.

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PLATE 1

EXPLANATION OF FIGURES

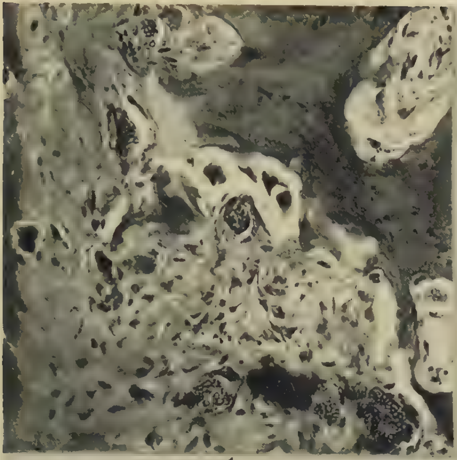
1 Section of symphysis of a female 56 days pregnant along the symphyseal face of the ischial ramus, showing three osteoclasts in a space between the ligament and bone. Note the numerous capillaries. Note small area of necrotic bone above and below osteoclasts. × 210.

2 Section of the same symphysis as above, showing the resorption of bone and invasion of the marrow cavity by fibrous tissue. Section shows a capillary communication between the vessels of the marrow cavity and those of the ligament. × 210.

3 Small area of the pubic ligament of a female 3 hours postpartum, showing extreme vascularity. Note the sections of the large blood sinuses above and below the bundles of collagenous fibers, and the capillaries ramifying through the fibers. This is a typical section—the whole ligament being highly vascular. × 210.

4 Section of the symphysis of a female 3 days postpartum, illustrating the extreme degree of bone resorption. The marrow cavity is completely filled with fibrous tissue, which is continuous with the bundles of fibers of the ligament, and the medial table of bone is removed from relatively extensive areas. ×100.

5 Small area of bone and adjacent ligament of the symphysis of a female 56 days pregnant, showing the process of resorption of bone by creeping replacement. Unstained area is dead bone, in which small invading islands of newly developed bone, surrounding single osteocytes, can be seen. × 430.



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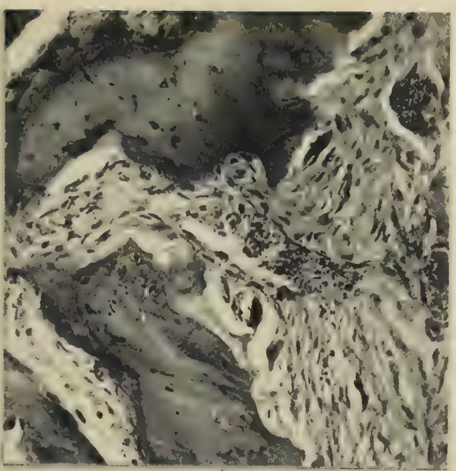

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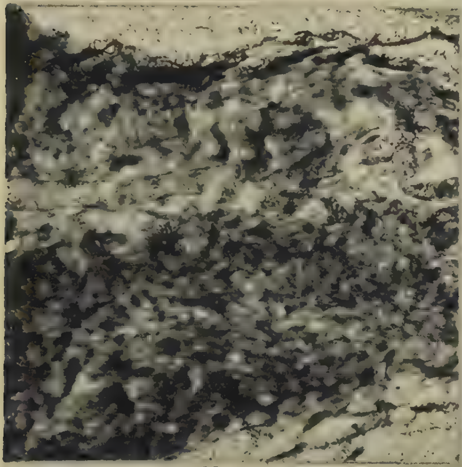

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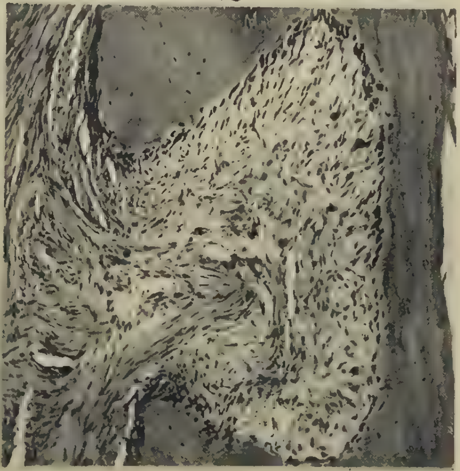

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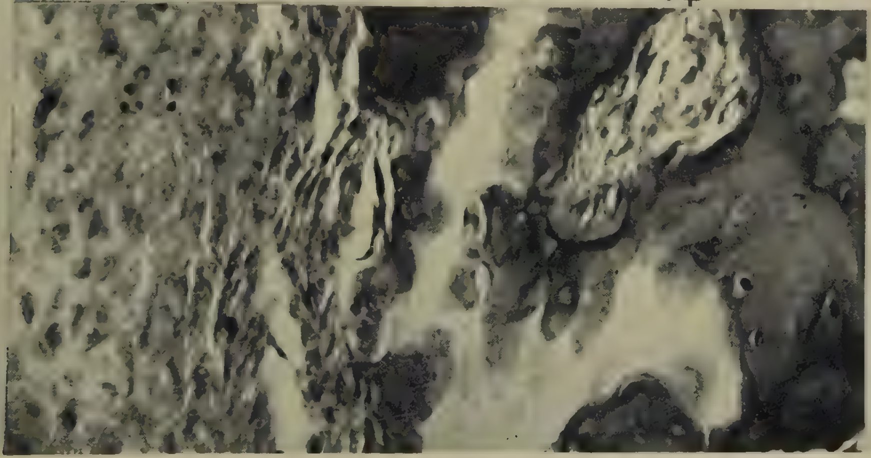

EXPERIMENTALLY INDUCED HYPERTHYROIDISM IN THE CHICK EMBRYO

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INTRODUCTION

It has been demonstrated by a number of investigators that under the influence of extracts of the anterior pituitary the avian thyroid can be caused to enter upon a state of hyperactivity (Noether, '32; Schockaert, '32; Severinghaus, '33). Such activity is characterized by marked increase in height of the follicular epithelium, accompanied by an increase in the number of mitoses, the disappearance of colloid and almost complete obliteration of the lumen. It has been observed in a number of instances, however, that in the bird as well as in other forms this condition is not maintained with prolonged administration of the thyrotropic hormone of the anterior pituitary (Seibert and Smith, '30; Loeb and Friedman, '31; Schockaert, '32; Anderson and Collip, '34; Collip and Anderson, '34; Hertz and Kranes, '34; Friedgood, '34). Involutional changes take place in the thyroid so that eventully it approaches its original condition. A number of theories have been advanced to account for these observations (refer to Loeb, '34; Collip, '35). It has recently been suggested, however, that the development of refractoriness is a function of the type of extract in which the hormone is administered (Werner, '36 a) and that immune responses to proteins probably play an important role (Werner, '36 b).

Previous experiments on the bird thyroid have utilized either hatched chicks or adults and have not been concerned with the embryo. The normal morphogenesis of the thyroid gland of the chick has been investigated a number of times

(Bradway, '29; Yoshikawa, '30; Sun, '32; Hopkins, '35). The present experiments represent an attempt to modify this normal development with a view to determining the effect on the embryo as a whole.

The writer is greatly indebted to Prof. A. B. Dawson, under whose direction this work was carried out.

MATERIALS AND METHODS

The embryos used in the present study were obtained from Barred Plymouth Rock eggs, incubated at a temperature of 38.2°C. The eggs were turned twice daily, both before and after treatment, to insure normal development. Chick embryos ranging in age from 4 to 20 days of incubation were used. The technique employed in a number of early experiments involved the administration of various substances by placing them in the air sac as described by Wislocki ('21), Hanan ('27) and Hurd ('28). The extracts included saline solutions of fresh sheep anterior pituitary, whole gland pituitary extract (sheep) (Parke, Davis and Company), and, as controls, Pannett-Compton ('24) solution as well as distilled water. These were administered either once or twice daily in amounts varying from 0.1 ml. to 0.5 ml. per injection. Histological examination of the thyroids after from 1 to 11 days of treatment revealed in all cases only the normal condition. The thyroids of those which were fixed after the eleventh day of incubation contained vacuolated colloid in quantities indistinguishable from treated and untreated controls. Furthermore, thyrotropic hormone of the anterior pituitary (Collip) administered in this manner to 13- and 14-day embryos induced no signs of hyperactivity on the part of the thyroids after 2 and 3 days of treatment.

The method of direct injection into the embryo was finally adopted. Each egg was candled to determine the position of the embryo, which was then marked on the surface of the egg. By means of a hack saw blade a small square window was made in the shell in that region of the air space which was nearest the embryo. A small portion of the outer shell membrane was then removed allowing a hypodermic needle to be inserted through the inner shell membrane and allantois. Injections were made directly into the embryo.

A number of solutions were tried in preliminary experiments, but the most successful cases of activated thyroids were obtained when thyrotropic hormone obtained from Dr. J. B. Collip was used. The quantity administered varied from 0.1 ml. to 0.6 ml. per injection in different experiments. It was found that embryos older than 9 days of incubation could withstand injections of 0.5 ml. twice daily.

Before fixation, each embryo was removed from the shell and carefully dissected free from the extra-embryonic membranes, after which its wet weight was determined. Thyroids, gonads, spleen, thymus and liver were dissected out. These, as well as the remainder of the embryo, were then fixed in Helly's modification of Zenker's fluid and preserved in 70% alcohol. The thyroids were embedded in paraffin, cut at 5 μ, and stained either with Harris' haematoxylin and eosin or with Mallory's triple connective tissue stain.

EXPERIMENTS

Series I

Embryos ranging in age from 12 to 20 days of incubation were injected with 0.2 ml. of thyrotropic hormone of the anterior pituitary (Collip) at 9 A.M. and at 5 P.M. and then were killed between 9 A.M. and 4 P.M. of the following day. All showed marked evidence of completely activated thyroids: high follicular epithelium, obliteration of the lumen and absence of alveolar colloid.

Control embryos, injected with either distilled water or Pannett-Compton solution, were found to have entirely normal thyroids when fixed after a corresponding length of time. A few 14- and 15-day specimens in both control and experimental groups contained abnormally swollen mesonephroi at the time of fixation. Sections indicated that the tubules in all portions of the organ were greatly distended, the walls having become thin and the cells flattened.

Series II

Embryos of 10 or 11 days of incubation were injected twice daily (9 A.M. and 5 P.M.) with 0.2 ml. of thyrotropic hormone of the anterior pituitary (Collip). On the following day, as well as on each succeeding day, two embryos were killed, the wet weights determined and the thyroids examined histologically. Complete activation was evident up to the eighteenth day, at which time early signs of regression began to appear. On the nineteenth day the follicular epithelium was definitely lower and some storage of colloid was taking place. The number of mitoses, although rather large, was still within the number frequently seen in untreated controls of 19 days' incubation. By the twentieth day of incubation the amount of colloid present had increased over that seen in the 19-day specimens, and the epithelium was more cuboidal. It was evident histologically that the thyroids were approaching the normal condition although it was possible to distinguish them from 20-day untreated controls. Wet weights of embryos receiving thyrotropic hormone remained consistently below those of treated and untreated controls (table 1). Examination of these embryos revealed that fat storage was below normal, especially in the neck region. This was accompanied by some emaciation.

TABLE 1

Comparison of wet weights of embryos injected with various solutions

AGE IN DAYS WHEN FIXED	NUMBER OF DAYS INJECTED	AVERAGE WET WEIGHT IN GRAMS			
		Thyrotropic hormone	Pannett-Compton solution	Distilled water	Uninjected controls
12.0	3	4.95	5.84	5.12	5.33
13.1	4	5.70	6.53	7.01	7.313
14.0	5	6.23	8.31	7.92	7.58
15.0	6	9.91	11.97	12.44	13.02
17.0	8	11.48	15.29	16.06	16.537
18.0	9	13.81	17.84	18.67	19.74
19.0	10	18.52	20.90	23.12	22.47

Embryos injected with distilled water and with Pannett-Compton solution served as controls. Normal thyroids were found in all cases, and the wet weights were not significantly different from normal embryos (table 1). Three specimens, two of which were killed on the fourteenth and one on the fifteenth day of incubation after 3 and 4 days of injecting respectively, contained hydronephrotic mesonephroi. One of the embryos killed on the fourteenth day had received distilled water, the other two embryos had received thyrotropic hormone.

DISCUSSION

The above experiments have demonstrated a number of points of interest with regard to the development of the chick under the influence of hyperthyroidism. The results indicate that the injection of thyrotropic hormone twice daily for more than 4 days causes a definite lowering of the wet weight of the embryo (refer to table 1). It was also observed that fat storage was less than in the controls, producing a slight degree of emaciation. No significant differences in sizes of organs and length of wing and leg segments were noted, however, when treated embryos were compared with controls. Earlier work has demonstrated that chick embryos show a certain amount of emaciation when thyroid grafts are placed on the chorio-allantois (Willier, '24). It was also found that the wing and leg segments were shorter, thyroid, gonads, etc., were smaller and that less fat was stored than in the controls.

Such hyperthyroidism is caused in the present experiments by a marked hyperactivity on the part of the embryonic thyroid itself, induced by the injection of the thyrotropic hormone of the anterior pituitary. This activation, as in adult birds (Noether, '32; Schockaert, '32; Severinghaus, '33), is represented by a high epithelium, almost complete obliteration of the lumen and an absence of colloid. Previous work has shown that in the chick colloid storage is normally initiated on the eleventh day of incubation (Bradway, '29; Yoshikawa, '30; Sun, '32; Hopkins, '35), a fact which has been confirmed during the present investigation. Prior to the eleventh day neither lumen nor colloid can be observed in the embryonic thyroid. Essentially this condition, accompanied by an increasingly high epithelium, can be maintained throughout most of the remainder of the incubation period by injections of thyrotropic hormone. During the first 7 days of treatment the follicular epithelium remains high and colloid storage does not occur. Beginning on the eighteenth or nineteenth day of incubation, however, a condition of refractoriness begins to manifest itself. These involutional changes in the embryonic thyroid are not a function of loss of potency on the part of the thyrotropic hormone in view of the fact that marked hyperactivity of the thyroids of previously untreated embryos was brought about in less than 24 hours by the injection of portions of the same solution. It is evident, therefore, that after activation, a thyroid regression similar to that reoprted by other investigators (Siebert and Smith, '30; Loeb and Friedman, '31; Schockaert, '32; Anderson and Collip, '34; Collip and Anderson, '34; Hertz and Kranes, '34; Friedgood, '34) for a number of adult forms can also be produced in the chick embryo by prolonged administration of thyrotropic hormone of the anterior pituitary.

A condition resembling the hydronephrosis described by Boyden ('24) has been observed in some of the embryos used in the present experiments. Abnormally swollen mesonephric tubules were present in a few 14- and 15-day chicks after the injection of either distilled water or thyrotropic hormone (Collip). An adequate explanation of these observations is impossible at the present time due to the fact that efforts to duplicate the result have not proved successful.

SUMMARY

1. Hyperthyroidism has been induced in chick embryos by the injection of thyrotropic hormone of the anterior pituitary.
2. Completely activated thyroids, containing a high follicular epithelium and showing an absence of colloid storage, were produced within 24 hours by direct injections into embryos ranging in age from 12 to 20 days of incubation. Equivalent

or larger quantities of the hormone were ineffective when placed in the air sac for the same length of time or for longer periods.

3. Involutional changes were observed in the thyroids of embryos receiving prolonged direct injection of thyrotropic hormone from the eleventh to the twentieth day of incubation. Such changes, while first evident on the eighteenth day, became marked on the twentieth day, and consisted of a lowering of the follicular epithelium and the reappearance of vacuolated colloid.

4. Embryos receiving prolonged treatment showed consistent loss in weight, diminution of fat storage and a slight degree of emaciation as compared with treated and untreated controls.

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CHOLESTEROL STORAGE AND BILE SECRETION IN CHORIO-ALLANTOIC GRAFTS OF LIVER

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ONE PLATE (THREE FIGURES)

INTRODUCTION

But few workers have investigated the problem of cholesterol storage in the liver of the chick embryo. Valentin (1871) in a generalized study of double refraction in tissues of the chick embryo found no evidence of it in the livers of embryos of 7, 9 and 11 days incubation. Hanes ('12) in a rather comprehensive study concluded that anistropic droplets of cholesterol esters first make their appearance in the liver of the chick embryo on about the fourteenth day of incubation. Such studies may determine the time during development when the liver first gives evidence of its function as a storage organ, but they obviously cannot supply evidence as to the time when this function is first established. It is possible that the storage function of the liver may have become established previous to the time when stored material is first identifiable under the microscope. An increase in the concentration of cholesterol in the blood stream may in part determine the time when this substance first becomes visible in the liver. To determine the time when the storage function first becomes established, livers from young embryos have been brought in contact with the blood stream of older embryos by means of the chorio-allantoic graft.

Sandstrom ('34) noted the constant occurrence of degenerative changes in grafts of hepatic tissue with a total incubation age of 12 or more days. She suggested that the inability of hepatic tissue to function in grafts leads to its degeneration. It seemed possible that functional differentiation may have occurred previous to the time of onset of these degenerative changes. To investigate this possibility, a series of grafts has been examined for the presence of exocrine secretory products.

MATERIAL AND METHODS

All embryos used in these experiments were of the Single Comb White Leghorn breed. Both the donor and host ages at the time of transplantation were varied so that the grafts might be grown on the membrane during the period when the concentration of cholesterol was at its maximum in the embryonic blood stream. The high point of the concentration of cholesterol in the blood is reached on the seventeenth day of incubation (Zorn and Dalton, '36). One series of grafts was fixed in 10% formalin. Frozen sections were than made in preparation for the histochemical analysis. A series of control embryo livers was treated in a similar fashion. To complete this analysis, another series of grafts was fixed in Champy fluid followed by post-osmication by the Kolatchev-Nassanov method.

OBSERVATIONS

Table 1 gives the results of the histochemical analysis of the droplets present in 10- and 18-day livers and in 10-day grafts removed from the chorio-allantois of 18-day hosts. As the table indicates, the droplets present in the graft livers give reactions identical with the droplets present in the 18-day livers. It should be mentioned that the majority of these droplets contain regions which give reactions with Nile blue sulphate and with Smith's stain. This suggests the presence of cholesterol, cholesterol esters, and cholesterol-fatty acid mixtures in the same droplet.

Table 2 lists and describes twenty-two of the more satisfactory grafts examined for the presence of anisotropic droplets. No grafts have been included in this table which show any evidence of degenerative changes. As the table demonstrates, anisotropic droplets first appear in grafts during the

TABLE 1[1]

REACTING MATERIAL	SUDAN III	ANISOTROPISM	OSMIC AFTER BICHROMATE	SOLUBILITY IN COLD		I_2-H_2SO_4 BROWN → GREEN	NILE BLUE SULPHATE	NEUTRAL RED	SMITH'S STAIN	FISCHLER'S STAIN
				Acetone	Absolute alcohol					
True fats	+++	—	+	+	+	—	Red	—	—	—
Cholesterol	—	+	—	+	+	+	—	—	—	—
Cholesterol esters	+	+	—	—	—	+	Reddish	—	—	—
Phosphatides	—	+[2]	—	—	+	—	Faint blue	+	+	—
Cerebrosides	—	+[2]	—	—	—	—	Faint blue	+	+	—
Phosopho-cerebrosides	—	+[2]	—	—	—	—	Faint blue	+	+	—
Cholesterol-fatty acid mixtures	+	+	+	+	+	+	Reddish	+ —	+	Cholesterol-oleic acid glycerin ester +
Fatty acids	—	—	—	—	+	—	Deep blue	+	+ —	+
Soaps	—	—	—	—	+	—	Deep blue	+	+ —	+
10-day liver	—	—	+ —	+	+	Faint +	Blue	—	Dark blue to black	—
18-day liver	++	+	Slight + mostly —	+	+ —	Strong +	Faint pink to —	—	— to faint blue-gray	—
10-day graft on 18-day host	++	+	Slight + mostly —	+	+ —	Strong +	Faint pink to —	—	— to faint blue-gray	—

[1] The sources from which most of the histochemical tests were obtained are Hanes ('12), Lee ('28) and Mallory and Wright ('24).

[2] Does not disappear on gentle heating.

seventh day of incubation (B-83), none being present previous to this time. Barely visible droplets on the eighth day, many increase in size during the next 24 hours, until on the ninth day, they approach the volume of the nucleus of the hepatic cell. The droplets in 10- and 11-day grafts equal the volume of those in normal 18-day livers. Figure 1 shows the size and distribution of the anisotropic droplets (changed to crystals after fixation in 10% formalin) as they appear in the 18-day liver. Figure 2 shows similar bodies in graft B-46, a 10-day graft on an 18-day host.

TABLE 2

GRAFT NUMBER	DONOR AGE IN HOURS	GRAFT AGE IN DAYS	HOST AGE IN DAYS	DAYS OF GRAFT ON MEMBRANE	ANISOTROPIC DROPLETS
B-101	80	6	15	3	None
B-102	72	6	15	3	None
B-111	80	6	15	3	None
B-112	80	6	15	3	None
B-113	80	6	15	3	None
B-114	80	6	15	3	None
B-77	60	6½	17	4	None
B-83	65	6⅔	17	4	Few and minute
B-109	72	6¾	16	4	Fairly numerous and minute
B-115	72	6¾	16	4	Fairly numerous and minute
B-100	72	7	16	4	Numerous and small
B-104	72	7	16	4	Numerous and small
B-105	72	7	16	4	Numerous and small
B-54	96	8	16	4	Numerous and small
B-58	72	8	17	5	Numerous and small
B-103	72	8	17	5	Numerous and small
B-107	80	8	17	5	Numerous and small
B-47	72	9	17	6	Numerous and large
B-55	72	9	17	6	Numerous and large
B-45	72	10	18	7	Numerous and very large
B-46	72	10	18	7	Numerous and very large
B-48	96	10	17	6	Numerous and very large

A series of livers from embryos varying in age from 9 to 19 days incubation were examined for the presence of anisotropic droplets. The first evidence of double refraction in this series was found in 11-day livers, more than 4 days after it first becomes apparent in grafts of livers on older hosts.

The presence of substances in the bile which will reduce osmic acid makes it possible to demonstrate the presence of secretory products in the bile canaliculi and in the gall bladders in liver grafts. Eighteen grafts varying from 8 to 13 days total age were fixed in Champy fluid and post-osmicated. All show the presence of secretory products in the bile canaliculi and also in the gall bladder when it is present. Figure 3 shows a portion of a section of graft B-20, a graft with a total age of 11 days, removed from a host of 19 days incubation. It shows the gall bladder filled with heavily osmicated material. This is evidence of very active secretion on the part of the liver cells of the graft.

DISCUSSION

Needham ('31) in referring to the work of Valentin (1871) states "Valentin investigated the doubly refracting droplets in chick embryos, and stated that none of the tissues of the 3rd, 4th, 5th or 6th day embryos showed them, but that on the 7th day a very few appeared. He obtained comparable results in a study of the embryonic liver of the frog." Actually Valentin found no clear-cut evidence of double refraction in the liver cells of the 7-day embryo. He states "die Anlage der beiden Leberlappen, ... lieferten kein deutliches Merkmal von Doppelbrechung." In the 9-day liver Valentin found 'Faserzellen' showing double refraction, but he makes no mention of the parenchymal cells. Further, in referring to double refraction in the 11-day embryo, he states "Die Massen der Leber und der Nieren führten zu keinen bemerkenswerthen Ergebnissen." Clearly Valentin found no evidence of anisotropic droplets in the liver cells of the chick embryo up to the eleventh day of incubation. From my histochemical study of the series of normal livers I have concluded that anisotropic droplets first make their appearance on the eleventh day of incubation. This is earlier than the time determined by Hanes ('12) but substantiates my previous finding (Dalton, '34). In addition to the cholesterol esters identified by Hanes, free cholesterol and cholesterol-fatty acid mixtures also appear to be present in the anisotropic droplets. This is true for the droplets present in the 18-day livers and in the 10-day grafts on 17- or 18-day hosts. This finding would appear to be evidence for the esterification of cholesterol by fatty acids in the hepatic cells, both in graft and in host livers.

In a study of the hepatic tissue of the chick embryo in chorio-allantoic grafts, Sandstrom ('34) found that degeneration of the hepatic cells occurred regularly, beginning on the twelfth day of incubation. She interpreted this phenomenon as being due to the inability of the liver cells to function in their abnormal environment. It has been shown above that liver cells in grafts are capable of the storage of cholesterol and that they possibly take part in its esterification. This storage first becomes evident in grafts during the latter part of the seventh day. In addition, I have found products of secretion in the bile canaliculi and in the gall bladder in liver grafts. The vacuoles described by Sandstrom and interpreted by her as evidence of beginning degeneration may well have contained cholesterol esters previous to fixation. Since embryonic liver cells have been shown to be capable of function on the chorio-allantoic membrane, it is suggested that the regularly occurring degeneration of hepatic tissue in grafts is due, indirectly, to the active functioning of the cells. Considerable degeneration of hepatic tissue follows complete obstruction of the common bile duct in humans (Lieber and Stewart, '34). Continued bile secretion in grafts in which there is no outlet, associated with an abnormal and possibly incomplete or insufficient blood supply, obviously would lead to degenerative changes. This possibility is supported by the observation of Sandstrom that degenerative changes occur more slowly when the gall bladder develops in liver grafts.

SUMMARY

1. Anisotropic droplets first appear in the chick embryo liver on the eleventh day of incubation.

2. In chorio-allantoic grafts of liver to older hosts, anisotropic droplets appear during the latter part of the seventh day.

3. The histochemical study of individual anisotropic droplets, in both hosts and grafts, indicates the presence in them of free cholesterol, cholesterol esters, and cholesterol-fatty acid mixtures. This suggests that esterification of cholesterol occurs in the hepatic cells of both hosts and grafts.

4. Cholesterol esterification and storage, and the presence of secretory products in the bile canaliculi are considered to be evidence for the functional activity of liver cells in chorio-allantoic grafts.

5. The regularly occurring degeneration of embryonic hepatic tissue in grafts is thought to be due, in part, to bile stasis resulting from functional activity in the absence of an outlet.

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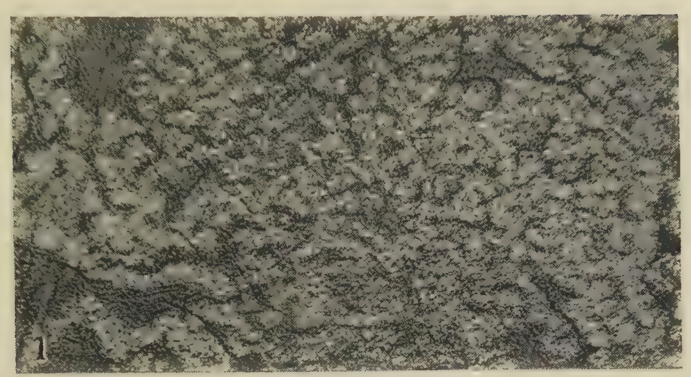
PLATE 1

EXPLANATION OF FIGURES

1 Frozen section of the liver of an 18-day host embryo viewed by polarized light, showing the size and distribution of the anisotropic crystals (cholesterol) present in the hepatic cells. Formalin; Nile blue sulphate. × 105.

2 Frozen section of the liver graft B-46, a 10-day graft on an 18-day host, viewed by polarized light. Note the relatively large crystals of cholesterol. Formalin; Smith's stain. × 105.

3 Section of liver graft B-20, an 11-day graft on a 19-day host showing a mass of heavily osmicated material in the gall bladder. Champy; post-osmication. × 520.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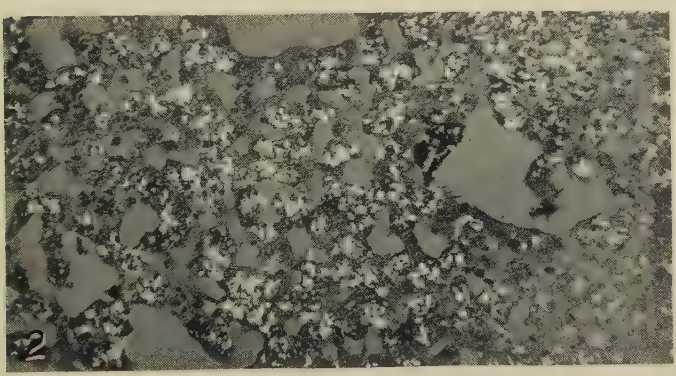
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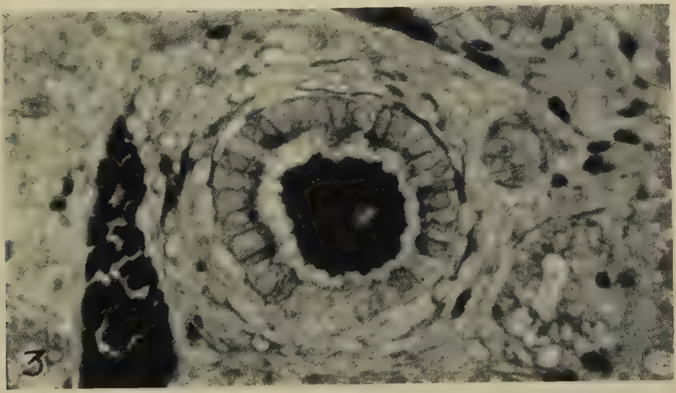
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THE DEVELOPMENT OF THE PARS INTESTINALIS OF THE COMMON BILE DUCT IN THE HUMAN FETUS, WITH SPECIAL REFERENCE TO THE ORIGIN OF THE AMPULLA OF VATER AND THE SPHINCTER OF ODDI

I. THE INVOLUTION OF THE AMPULLA

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EIGHT TEXT FIGURES AND FIVE PLATES (FIFTEEN FIGURES)

The present investigation is one of a series of studies being carried on jointly in the Department of Anatomy and clinical departments of the University Hospital with the object of throwing more light on the pathologic and normal physiology of the human biliary tract.

Among these problems, the regulation of the flow of bile at the duodenal orifice of the common duct occupies a central position. Thus, it having been demonstrated quantitatively, in one of these studies, that evacuation of the human gall bladder is markedly retarded during pregnancy (Gerdes and Boyden, '36) all the evidence seems to indicate that the cause of biliary stasis is to be attributed to a hyper-stimulation of smooth muscle fibers surrounding the intra-mural portion of the common bile duct. Yet a study of the literature reveals no unanimity as to the nature of this investing musculature. One school of workers has maintained that the occluding mechanism is merely the muscle tunic of the intestine operating on a duct that passes obliquely through its wall. Others have held that the physiological as well as the anatomical evidence suggests the presence of an intrinsic choledochal musculature, influenced, perhaps, by the movement and tone of the duodenal wall, but structurally and, in the main, physiologically independent of the gut.

The question may well be asked, in view of the long line of anatomical investigations dating from the seventeenth century, why there should be any difference of opinion as to whether or not the duodenal end of the bile duct is provided with a specific musculature. The answer would seem to be as follows: Until recently, only two anatomical techniques have been employed: a) The serial section method, first applied to the problem by Gage (1879) then Oddi (1887) and others; and b) the maceration method, first applied by Oddi (1887), then Hendrickson (1898) and others. Each method has had distinct limitations. Owing to the complexity of the adult structures, serial sections could not be used to make wax or other three dimensional reconstructions, and without the latter the pattern was too complicated to be visualized. Furthermore, many investigators have failed to use specific smooth muscle stains. The maceration methods, on the other hand, have involved destruction of part of the evidence, and when completed have left only miniature specimens in which important details were necessarily missing or obscure. As a result, the basic consideration of structure has been left to inference drawn from physiologic experiments. Babkin's comment on the situation ('28) is that "a new anatomical investigation with new methods is emphatically to be desired."

Accordingly, we have attempted a third approach; namely, the reconstruction of successive stages in the development of the duct—a method which has made possible the separation of intrinsic choledochal from intestinal tunics in point of time and then the display of essential relations before the muscle layers have become too complicated to model.

For purposes of convenience the presentation is divided into three parts, one covering the changing intramural pattern of the ducts, the others the origin and arrangement of the musculus proprius—that investing sheath of fibers which was first described by Glisson (1654) and which is now generally known by the name, Sphincter of Oddi.[1]

MATERIAL AND METHODS

As indicated in table 1, the essential material consists of fourteen serially sectioned embryos and fetuses from the Minnesota Embryological Collection—specimens ranging from 11.0 to 65.0 mm. in length (crown-rump measurement)—and four serially sectioned duodenums from freshly obtained fetuses ranging in length from 155 to 525 mm. (crown-heel measurement). In addition, one of us (E.A.B.) has had the privilege of examining sectioned human embryos in the Carnegie, Cornell and Harvard collections.

The four oldest specimens (secured by R.A.S.) were obtained from the delivery room either at the time of death of the fetus or within 5 or 10 minutes thereafter. The duodenum was carefully dissected out, fixed in Susa's fluid according to the Heidenhain method, cut serially in paraffin at 10 μ and stained with the Mallory-Azan technique—the stain which best differentiates smooth muscle from connective tissue. In so far as possible the plane of section was made to lie at right angles to the long axis of the duodenum.

After all the embryos had been studied serially, four critical stages (15-, 18-, 33- and 115-mm. specimens) were selected for reconstruction in wax, according to the Born method. For older fetuses (365-, 430- and 525-mm. specimens) the method of graphic-reconstruction was employed. In this technique the duodenal wall on the side opposite the ampulla was used as a base line and the outlines of ducts and ampulla plotted in with the aid of a filar micrometer. By these two methods it was possible to cover the entire period of development before birth.

OBSERVATIONS

A critical survey of this graded material reveals two striking changes that are undergone by the terminal portion of

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[1] For review of older literature and origin of technical terms, see Boyden ('36).

TABLE 1

Growth changes in the pars intestinalis of the common bile duct and ampulla

EMBRYOS[1]		SIZE OF FENESTRA[2]	LENGTH OF PORTIO DUODENALIS			INTESTINAL ORIFICES OF AMPULLA VATERI			
No.	Length (C.R.)		Junct. of bile and panc. ducts	From junction to ost. sup.	Per cent ampulla	Superior ostium	Inferior ostium	Micra apart	Rel. to bil. flex.
	mm.	μ		μ					
H-68	11	..	(Muscle not formed)	25	..	Duct empties into primary lumen			
H-305	15								
sag.		100	Just outside fenestra	..	..	Ends blindly in vacuole of duod.			
H-260	18	150	Just outside fenestra	30	100	Into dext. cav.	Into sin. cav.	75	Just inf.
H-265	21	140	At fenestra	140	100	Into sin. cav.	Into dext. cav.	60	..
H-273	20	150	At fenestra	105	100	Into dext. cav.	Present, but not patent	..	..
H-15	22	168	Just inside fenestra	108	100—	Into defin. cav.	Present, but not patent	..	..
H-326	25	135	Just inside fenestra	120	100—	Into defin. cav.	Present, but not patent	..	..
H-29	26	144	12 μ below inf. marg.	120	90	Two ostia	Present, but not patent	..	Just sup.
H-98	30								
sag.		..	..	..	..	Two ostia	One ostium	..	Just inf.
H-16	33	120	30 μ below	195	87	One ostium	One ostium	315	Just sup.
H-313	35	160	20 μ below	120	90	One ostium	One ostium	400	..
H-12	41	150	Just below	225	100—	One ostium	One ostium	..	Just sup.
H-100	43								
sag.		..	..	..	..	Two ostia	One ostium	..	Just inf.
H-55	65	350	100 μ below	400	75	One ostium	One ostium	..	Just sup.
F-4	115 C.R. 155 C.H.	200	430 μ below	0.940 mm.	69	One ostium	Not patent	..	Just sup.
F-3	365 C.H.	1.38 mm.	1.51 mm. from inf. marg.	1.62 mm.	52	One ostium	One ostium	1.00 mm.	Just inf.
F-1	430 C.H.	1.20 mm.	1.49 mm. from inf. marg.	1.94 mm.	57	Only one opening on plica			Just sup.
F-2	525 C.H.	2.10 mm.	0.73 mm. from inf. marg.	3.64 mm.	83	Only one opening on plica			..

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[1] Serial sections cut transversely unless otherwise noted; italics indicate that bile ducts, mucosa and musculature were reconstructed graphically or in wax.

[2] Vertical distance between superior and inferior margins of window in circular muscle of duodenum.

the bile duct: First, the acquisition of two or more outlets into the cavity of the duodenum, as a result of which the ampulla acquires a segment of intestinal origin; second, the involution of the ampulla—a retrograde process which plays a decisive role in segregating the musculus proprius of the ducts from the tunica muscularis of the duodenum. Before discussing these two processes in detail it is necessary to establish the position of the ampulla in the embryonic gut.

The relation of the plica longitudinalis to the flexures of the duodenum. In all embryos in which the plane of section was favorable, the orifice of the bile duct was found to be associated with a constant but hitherto undesignated bend in the fetal duodenum. This we have named (E.A.B.) the biliary flexure.[2] As indicated in table 1, the ampulla empties just below this bend in four cases (e.g., fig. 13) and just above it in six other cases (e.g., fig. 15).[3]

Probably Glisson (1654) was the first to note this flexure in the adult when he wrote, ‘‘The common duct . . . . proceeds straight downwards towards the end of the duodenum or (if you prefer) the beginning of the jejunum; for this means the same . . . .’’[4] Since then, this biliary flexure has been well figured by Luschka (1863), by Jonnescu and Charpy (in Poirier and Charpy, ed. of '01) and by recent texts of anatomy (e.g., Jackson's edition of Morris, '33). Moreover it frequently appears in roentgenograms. Undoubtedly it fails to appear in certain individuals, but our observations in the dissecting room would lead us to believe that it persists in a large per cent of the population.

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[2] Flexura biliaris. In accordance with the suggestion made by Professor Ogle of the Latin Department we have used the adjective ‘biliaris’ (after the analogy of the French word biliaire) in preference to the form ‘bilarius’ employed by Bidloo (1685).

[3] In the adult, Stracker ('09) has noted another kind of variability in position, namely a variation in the distance of the major papilla from the pylorus. This ranges from 55 to 142 mm., the individual cases being grouped as follows:

Distance in millimeters	60–69	70–79	80–89	90–99	100–109	110–119	120–129	130–139
Number of cases	1	6	11	7	7	8	6	2

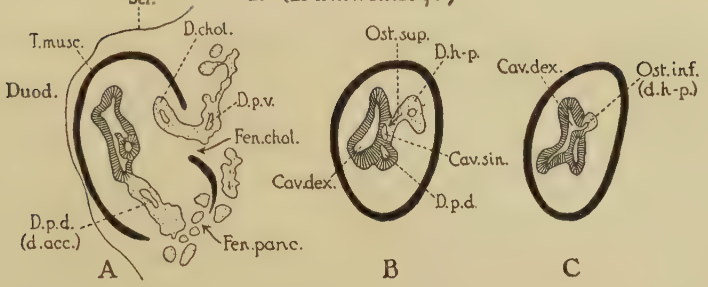
[4] See footnote 7 and page 22, Boyden ('36).

The acquisition of multiple openings into the duodenum. The clue to this phenomenon is to be found in the way in which the cavity of the gut tract is reestablished following the proliferative stage in the development of the duodenum. It will be recalled that soon after the 5-mm. stage (Lewis, '12; Rietz, '17) the hollow epithelial primordia of the gall bladder, cystic duct and common bile duct become solid but that by the 7.5-mm. stage a lumen appears in the common bile duct. About this time, also, the epithelium of the duodenum begins to proliferate but only to the extent that its cavity becomes broken up into discontinuous vacuoles.

At the 15-mm. stage, represented in our series by a sagittally sectioned embryo (figs. 9 to 11), the epithelial anlage of the gall bladder is still solid, the common bile duct has a well-defined lumen, but the channel of the duodenum has not yet become reestablished. (For photomicrographs of this specimen, see figs. 9E and 11, Boyden, '35). A wax case of these discontinuous cavities reveals the striking fact that the hepato-pancreatic duct empties into an isolated vacuole (*D. h.-p.*, fig. 10). Already, however, the vacuoles at the upper end of the duodenum are beginning to coalesce to form two channels (figs. 9 and 10)—a larger dextral cavity (on the greater curvature of the duodenum) and a smaller sinistral one (on the lesser curvature).

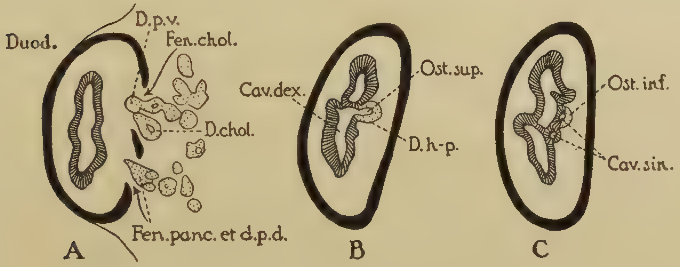
In the next older embryo of our series (18 mm.) the coalescence of cavities is more pronounced (fig. 14) but here a new feature has appeared. By fusion of vacuoles the hepato-pancreatic duct has acquired two channels into the duodenum: A superior one (*Ost. sup.*), opening into the dextral cavities of the gut and an inferior one (*Ost. inf.*), 75 μ below the other, which opens into the sinistral cavity. A similar situation is shown in another embryo of this age except that the order of cavities is reversed (fig. 1 B and C); but in a third specimen the inferior channel has failed to become patent (fig. 2 C). Slightly later the right and left cavities coalesce, leaving the duodenum with a single crenulated lumen (figs. 3 and 4). Yet in spite of this the orifice of the hepato-

1. (21 mm. embryo)



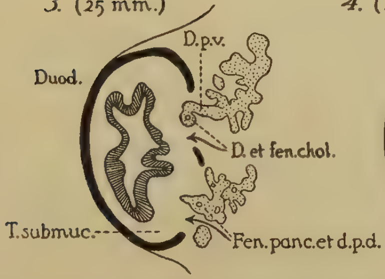
Ser.
T.musc.
Duod.
D.chol.
D.p.v.
Fen.chol.
D.p.d.
(d.acc.)
Fen.panc.
A
Ost.sup.
D.h-p.
Cav.dex.
Cav.sin.
D.p.d.
B
Cav.dex.
Ost.inf.
(d.h-p.)
C

2. (20 mm. embryo)



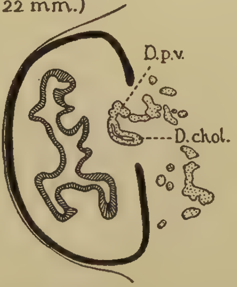
Duod.
D.p.v.
Fen.chol.
D.chol.
Fen.panc. et d.p.d.
A
Cav.dex.
Ost.sup.
D.h-p.
B
Ost.inf.
Cav.sin.
C

3. (25 mm.)



Duod.
D.p.v.
D. et fen.chol.
T.submuc.
Fen.panc.et d.p.d.

4. (22 mm.)



Figs. 1 to 4 Transverse sections through the duodenum of four human embryos from the Minnesota collection. Magnification × 40. These sections illustrate: 1) The relation of the junction of the common bile duct and the duct of the ventral pancreas to the aperture in the tunica muscularis; 2) the relation of the upper and lower orifices of the hepato-pancreatic duct to the two lumens of the duodenum. 1. Series no. 265: *B,* 7 sections below *A; C,* 3 sections below *B.* 2. Series no. 273: *B,* 7 sections below *A; C,* 3 sections below *B.* 3. Series no. 326. 4. Series no. 15. *Stippled* areas, pancreas; *lined* areas, epithelium of gut; *solid* black areas, muscle of duodenum. *Cav. dex., Cav. sin.,* right and left lumens of duodenum (compare fig. 14); *D. chol.,* common bile duct; *D.p.d., D.p.v.,* ducts of dorsal and ventral pancreas, respectively; *D.h.-p.,* hepato-pancreatic duct; *Fen. chol.,* 'window' in tunica muscularis for passage of common bile duct and duct of ventral pancreas; *Fen. panc.,* 'window' for passage of duct of dorsal pancreas (accessory pancreatic duct); *Ost. sup., Ost. inf.,* upper and lower orifices of primitive ampulla (hepato-pancreatic duct); *Ser.,* peritoneum of duodenum.

pancreatic duct usually remains double for some time (see columns 6 and 7, table 1).

By the 33-mm. stage the growth of the intestine has carried the two orifices so far apart (315 μ) that the forked channels are clearly visible from the submucosal side of the model (*Ost. sup.* and *inf.*, fig. 16). A similar process has taken place in the accessory pancreatic duct, except that the inferior opening in this specimen has not broken through (see insert, fig. 16). Transverse sections of the same specimen (figs. 17 to 19) show clearly that the portion of the duct lying between the two orifices remains in contact with the mucosal epithelium of the gut and the same is true of the hepato-pancreatic duct, thus revealing the way in which multiple openings may arise. Figure 19 is of special interest as showing how the wall of the superior channel of the hepato-pancreatic duct is composed of two segments—a proximal one, which from its lighter staining reaction is obviously of ductular origin, and a distal one, more darkly stained, which must be derived from the vacuolated cavities of the duodenum. This points strongly to a participation of the intestinal epithelium in the formation of the ampulla of Vater, and may explain why goblet cells are so numerous in the ampulla of the fetus, although such cells may also be found above the hepato-pancreatic junction, in the lower intramural portion of the bile and pancreatic ducts.

At this stage no longitudinal fold or papilla is visible on the mucosal surface of the model. Helly's statement ('00) that the papilla is present in a 28.5-mm. embryo is probably based on the deceptive appearance of villi as seen in histological sections. This also explains another confusing statement of his, namely, that the pancreatic and bile ducts of this embryo unite inside the papilla.

Summarizing these embryonic stages, it can be stated that eight out of twelve available human embryos between 18 and 65 mm. in length exhibit hepato-pancreatic ducts with both superior and inferior orifices—three of them, indeed, with multiple orifices (table 1). In the remaining four both channels are present but only the upper one is patent throughout. When we turn to the last 3 months of pregnancy, however, only one fetus out of three exhibits a double orifice of the ampulla, the two openings in that specimen being about 1 mm. apart (figs. 6, 21 and 23).

In the adult, the incidence of multiple openings is still further reduced, Stracker ('09) having found only five cases in 140 cadavers. In one of these the two orifices of the ampulla were 3 mm. apart and in another they were located on separate wart-like projections of the plica longitudinalis.[5]

Our specimens, however, do not substantiate Stracker's hypothesis that the presence of two or more openings can be explained on the basis of Helly's theory that the intestine grows faster than the opening of the bile duct and so draws it out into a longitudinal cleft which, closing from below upwards, leaves a frenulum below and an orifice above, or several openings if its closes irregularly. Rather, our models indicate that multiple openings arise as a result of the peculiar method by which the lumen of the embryonic duodenum is reestablished and that, normally, the ampulla of Vater acquires two openings into the gut. Of these two, the lower one is usually suppressed (table 1), but it may persist in the form of a blind pocket, a wart-like projection, a spilt frenulum or a patent orifice.

The involution of the ampulla of Vater. At the time that the primordia of the liver and ventral pancreas first appear, there is no such thing as an ampulla of the ducts; the ventral pancreas is merely an outgrowth of the duodenum adjacent to the point where the latter gives rise to an hepatic diverticu-

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[5] Apparently the first case in the literature is that reported by Vater in 1720 (see p. 229, Boyden, '36), but Stracker has provided the most detailed study of these anomalies, as, indeed, of the plica longitudinalis itself. He has named the thicker upper end of it the 'corpus plicae' and retained Santorini's term 'frenulum' for its caudally appended fold. In twenty-eight out of fifty-one fetal specimens ranging from 7.2 to 60.0 cm. long he found that the fold had the shape of a longitudinal swelling with parallel sides. In another seventeen, it took the form of a short, cone-shaped papilla and in six other specimens it consisted of a short papilla that bulged at its middle. He showed that the frenulum was often split, in younger fetuses, but that the edges tended to grow together with age.

lum. But about the 8-mm. stage (compare figs. 15 and 16, Boyden, '26), the zone where the ducts of these two organs meet the duodenum elongates into a segment that is common to both channels. This new structure is the hepato-pancreatic duct or what may be called the primitive ampulla. Also, about this time it shifts its axis from the horizontal to an inclined plane, so that from this stage on, the bile passage will traverse the outer coats of the duodenum obliquely. By the 15-mm. stage (fig. 11), further elongation of this segment has pushed the junction of bile and pancreatic ducts out to the level at which the intestinal muscle is forming. Therefore, at first, the

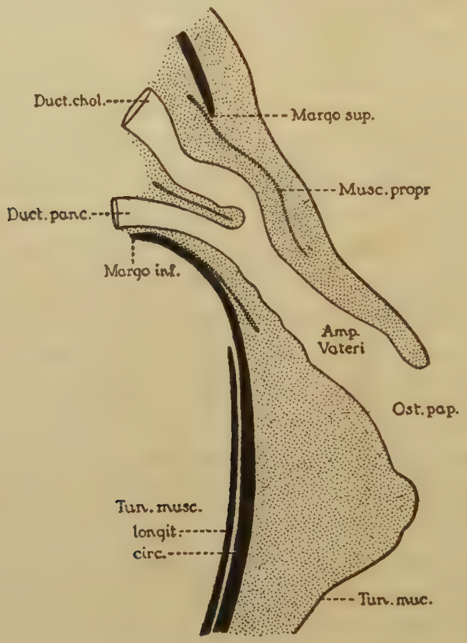

Fig. 5 Sagittal reconstruction of pars intestinalis of common bile duct in a 15.5 cm. (C. H.) fetus, no. F-4. Stimated age, 16 weeks. Magnification × 35. *Margo sup.* and *inf.*, upper and lower margins of the fenestra choledocha, the 'window' or hiatus in the muscle layer through which the ducts enter the wall of the gut. Note that junction of ducts has receded a fourth of the way through the wall. *Musc. propr.*, intrinsic muscle of ducts (heavy dots) which is differentiating in situ from mesenchyma; *T. muc.*, luminal surface of mucosa; *Ost. pap.*, orifice of ampulla of Vater.

pars intestinalis (s. portio duodenalis) of the bile passages consists wholly of a primitive ampulla.[6]

Beginning about the 20-mm. stage, however, the point of junction begins to be drawn back through the window in the intestinal muscle (*Fen. chol.*, figs. 1 to 4) and soon comes to lie within the submucosa of the intestine (33-mm. stage, figs. 15 and 18). At the beginning of the fourth month (fig. 5)

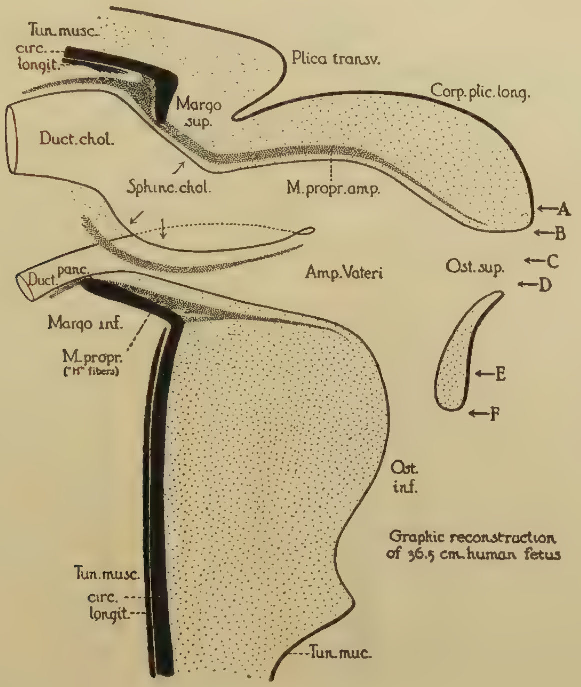
Tun.musc.
circ.
longit.
Plica transv.
Corp.plic.long.
Margo sup.
Duct.chol.
Sphinc.chol.
M.propr.amp.
A
B
Amp.Vateri
Ost.sup.
C
D
Duct.panc.
Margo inf.
M.propr. ("H" fibers)
E
F
Ost. inf.
Graphic reconstruction of 36.5 cm. human fetus
Tun.musc.
circ.
longit.
Tun.muc.

Fig. 6 Sagittal view of ampulla of Vater in a 36.5 cm. (C.H.) fetus, no. F-3. Estimated age, 29 weeks. Magnification × 17.5. This specimen illustrates the persistence of both upper and lower orifices of the ampulla. (For level C, see fig. 21; for level F, see fig. 23). The lower orifice lies presumably in the frenulum, the upper orifice in the corpus of the plica longitudinalis. The reason why the ampulla appears to lie at right angles to the intestine instead of obliquely is because the duodenum is bent at this place (see biliary flexure, fig. 13). Heavy dots show position of intrinsic musculature.

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[6] As soon as it acquires its new orifices (and, thereby, its duodenal component) it can be called the definitive ampulla.

the zone of junction of the two ducts is a fourth of the way through the wall and as the fetuses approach term it has receded halfway to the mucosa (two out of three cases; see figs. 6 and 7; also column 5, table 1).

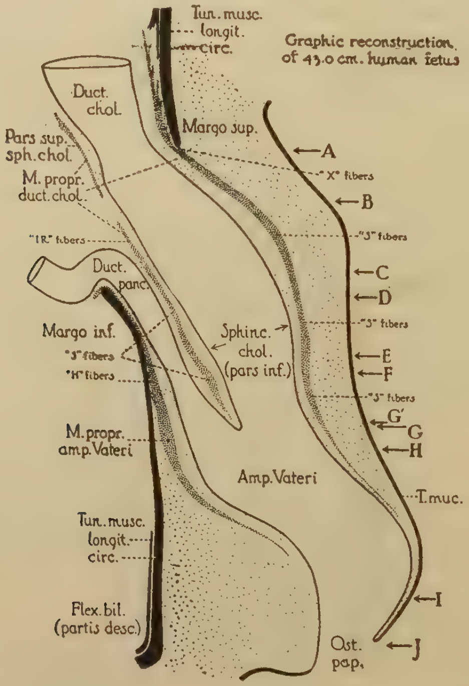
Tun. musc.
longit.
circ.
Graphic reconstruction of 43.0 cm. human fetus
Duct. chol.
Pars sup. sph. chol.
M. propr. duct. chol.
Margo sup.
A
"X" fibers
B
"IR" fibers
"S" fibers
Duct. panc.
C
D
"S" fibers
Margo inf.
"S" fibers
"H" fibers
Sphinc. chol. (pars inf.)
E
F
"S" fibers
G'
G
H
M. propr. amp. Vateri
Amp. Vateri
T. muc.
Tun. musc.
longit.
circ.
Flex. bil. (partis desc.)
I
Ost. pap.
J

Fig. 7 Sagittal view of ampulla of Vater in a 43-cm. fetus, no. F-1. Estimated age, 34 weeks. Magnification × 17.5. Note that junction of bile and pancreatic ducts now lies halfway between fenestra and orifice. *Flex. bil.*, biliary flexure of pars descendens of duodenum; *heavy dots*, intrinsic musculature of ducts (to be discussed in next article; 'H' fibers, 'S' fibers, etc., zones described by Hendrickson, 1898). *A–J*, transverse levels (see Article III).

Between birth and maturity it must undergo still further reduction, for Mann and Giordano ('23) have shown that in 76% of autopsies (152 out of 200) the ampulla of Vater was only 2 mm. or less in length, an insignificant portion of the stretch of 15 to 20 mm. that constitutes the intramural portion of the duct (Luschka, 1869). Job's figures ('26) are even more extreme, that author having found an ampulla in only eight out of 151 cadavers.[7]

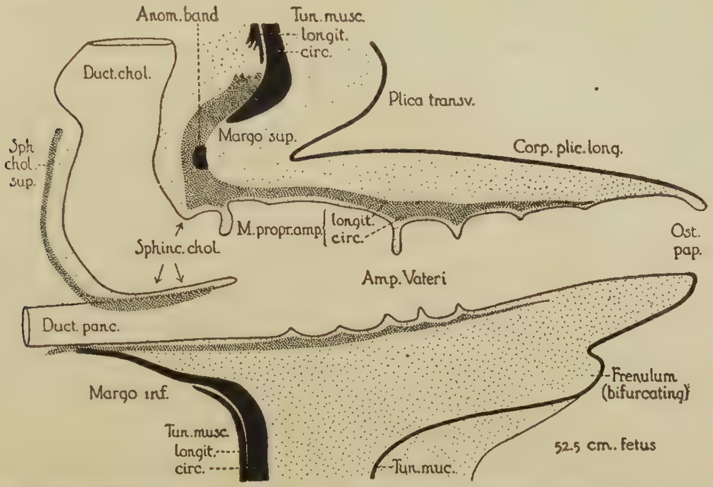
Anom. band
Tun. musc.
longit.
circ.
Duct. chol.
Plica transv.
Sph chol. sup.
Margo sup.
Corp. plic. long.
M. propr. amp.
longit.
circ.
Sphinc. chol.
Ost. pap.
Amp. Vateri
Duct. panc.
Frenulum (bifurcating)
Margo inf.
Tun. musc.
longit.
circ.
52.5 cm. fetus
Tun. muc.

Fig. 8 Sagittal view of ampulla of Vater in a 52.5-cm. fetus, no. F-2. Estimated age, 8 to 9 months. Fetus hydrocephalic with spina bifida and bilateral club foot. Magnification × 17.5. Heavy dots, intrinsic musculature of ducts.

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[7] The first satisfactory statistical study of the ampulla must be attributed to Mann and Giordano ('23). Such previous data as Schirmer's (1893), Letulle and Nattan-Larrier's (1898), Opie's ('03), Baldwin's ('11), Belou's ('15) and McWhorter's ('21) contain either incomplete, misleading or incidental figures.

Mann and Giordano sought to demonstrate in what per cent of individuals the ampulla was long enough to permit regurgitation of bile into the pancreatic duct, assuming that such a process could be caused by obstruction of the papilla or spastic contraction of a possible sphincter papillae. Their careful dissection of 200 consecutive necropsies revealed four types of orifices.

	Number of cases	*Per cent*
I. Bile and pancreatic ducts open separately.	62	31
II. Both ducts open into a shallow ampulla 2 mm. deep.	90	45
III. Both ducts open into an ampulla 3 to 10 mm. deep. Twenty-eight ampulla (14% of 200 necropsies) were 3 mm. long; five (2.5%) were 4 mm. and the remaining seven 3.5%) ranged between 5 and 10 mm.	40	20
IV. Duct of Wirsung absent or reduced to fibrous cord.	8	4
	200	

Adding together the data given in classes II and III, it appears that the average depth in all specimens having an ampulla is only 2.5 mm. These data are quite different from the figures supplied by Nagai and Sawada ('25). In thirty-four Japanese adults, the average depth of the ampulla was reported as 5 mm. and ranged from 2 to 9 mm. as follows:

Millimeters long	2	3	4	5	6	7	8	9
Number of cases	3	7	4	10	3	1	4	2

These figures suggest that perhaps there is a racial difference between Japanese and occidental races, but the samples are too small to be more than suggestive.

This striking fact being established, what is the process by which the ampulla is reduced? Is it, perchance, the same as that which subdivides the human cloaca into urogenital and rectal channels (i.e., a progressive downgrowth of a connective tissue septum), or is the retrogression of the ampulla merely apparent, i.e., a consequence of the more rapid growth of the surrounding wall of the duodenum? Some such explanation would seem to be more in accord with the facts, for when the ampulla is measured it appears that its absolute length increases with age (column 4, table 1)—from approximately 0.1 mm. at the 20-mm. stages, when involution first begins, to 1.6 to 3.6 mm., in the last 2 months of intrauterine growth.[8]

This apparent retrogression of the ampulla, however, has great practical significance; for, as Mann and Giordano have already pointed out, pancreatitis resulting from a regurgitation of bile into the ductus pancreaticus is theoretically possible only in that very small per cent of individuals in whom the ampulla is long enough to lodge a small stone or to permit the ampullary musculature or some other obstructing agency to come into play. Why the ampulla continues to grow in some individuals and not in others, postnatally, cannot be answered by the material at hand.

Another consequence of the different rate of growth of intestinal wall and ampulla is its effect on the position of the sphincter choledochus, the intrinsic musculature that surrounds the terminal portion of the bile duct. It carries it away from the level of the window in the intestinal muscle, where it is first differentiated from mesenchyme (compare figs. 17 and 18) and deposits it deep in the submucosa where its independence of the intestinal musculature can be readily demonstrated—a condition quite different from that which obtains in most other mammals (Boyden, '37).

Finally, of what use is the human ampulla? Our conclusion is that its chief function is to give rise to the terminal valvules that guard the orifice of the bile and pancreatic ducts, thereby preventing the regurgitation of intestinal contents. In our specimens these valvules first appear as wrinkles in the lining epithelium of the ampulla, between the third and fourth fetal month (see model of 115-mm. (C.R.) fetus in next article). By the sixth fetal month they have developed into low rounded folds (figs. 21 and 22).[9]

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[8] For the most probable explanation of its reduction in the human embryo see section on 'gradients,' in next article of this series.

SUMMARY

The development of the ampulla of Vater has been followed in eighteen human embryos and fetuses ranging from the 11-mm. stage to term. Four of the younger stages have been modelled in wax and three of the older ones rendered by graphic reconstructions.

In these specimens the plica longitudinalis (major papilla) is located just above or just below a constant bend in the pars descendens which is herewith named the biliary flexure of the duodenum.

Early in development the primitive ampulla (hepato-pancreatic duct) acquires two (or more) openings into the intestine. These new channels arise through the coalescence of vacuoles following the nearly solid stage in the development of the duodenum. The duodenal epithelium is thus caused to participate in the formation of the ampulla. The lower of the two orifices is usually suppressed but may persist in the form of a blind pocket, a split frenulum or even a second patent opening.

The ampulla, itself, is at first coextensive with the oblique intramural passage of the duodenum, but beginning with the 20-mm. stage the point of junction of bile and pancreatic ducts recedes from the window in the intestinal wall into the submucosa, until, near term, it lies halfway between the tunica muscularis and the end of the papilla. This retrogression is apparent rather than real, since the ampulla increases in

[9] As pointed out in a previous study (Boyden, '36), Vesalius was probably the first to note these folds in the adult, but they were not described in detail before the time of Santorini (1775). Stracker ('09) has given the best recent description of them.

absolute length throughout intrauterine development. The cause of its apparent decrease in length will be considered in the next article of this series.

The practical effect of this involution of the ampulla is to virtually eliminate the theory that pancreatitis is due to regurgitation of bile into the pancreatic duct following obstruction of the ampulla. Incidentally, the recession of the ampulla into the submucosa of the intestine makes it possible to demonstrate, in man, the independence of the choledochal musculature. Short as it is, however, the human ampulla subserves one important function: It gives rise to valvules which guard the orifice of the ducts and so prevents the regurgitation of the intestinal contents.

The second article in this series will describe the early development of the sphincter of Oddi.

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PLATE 1

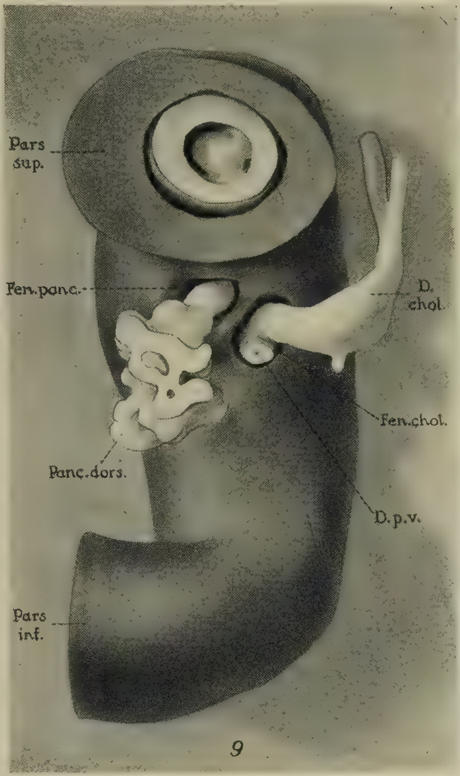
EXPLANATION OF FIGURES

Wax models of pars descendens duodeni of a 15-mm. human embryo (H-305), illustrating relation of ducts to 'windows' in tunica muscularis and to vacuolated cavity of duodenum. Magnification × 90.

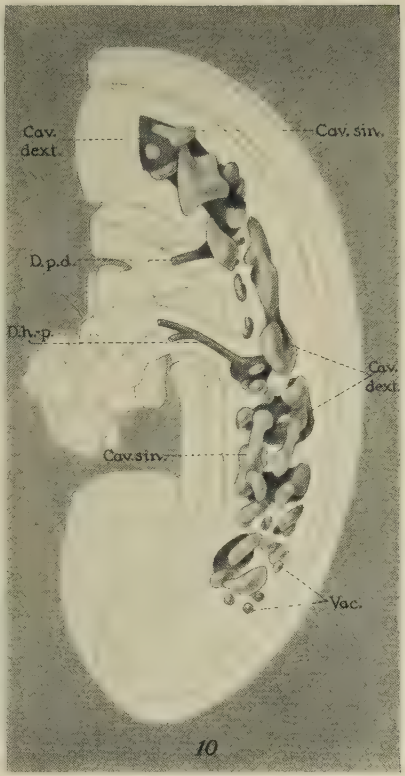
9 Duodenum viewed from left side, its muscle modelled in dark wax, epithelium and ducts in white wax. For position in embryo, see figures 9 E and 11, Boyden ('35). *D. chol.*, common bile duct; *D.p.v.*, duct of ventral pancreas; *Fen. chol.*, fenestra for bile duct and associated pancreatic duct; *Fen. panc.*, 'window' for accessory pancreatic duct (of dorsal pancreas).

10 Wax cast of cavity of duodenum—same orientation as in figure 11. Note that the lumen is broken up into discontinuous vacuoles but that the latter have begun to coalesce to form the two channels shown in figure 14. *Cav. dex.*, the cavity which in the next stage will become the right lumen of the duodenum; *Cav. sin.*, left lumen of later stages (compare figs. 1 to 4); *D.h.-p.*, hepato-pancreatic duct; *D.p.d.*, duct of dorsal pancreas.

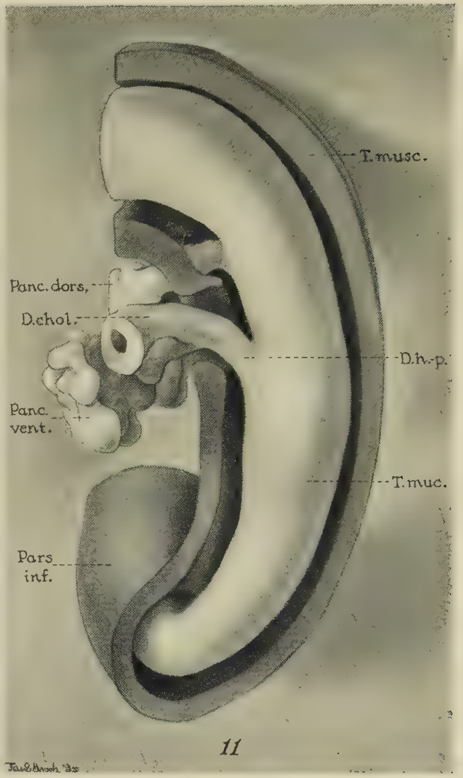
11 Duodenum viewed from posterior side. Note that primitive ampulla (*D.h.-p.*) joins duodenum at acute angle (thus early establishing the obliquity of the pars intestinalis); also observe that the junction of the bile and pancreatic ducts lies just outside the aperture in the tunica muscularis, so that in the beginning the ampulla is coextensive with the oblique passage through the intestinal wall. *T. muc.*, tunica mucosa of duodenum.


Pars sup.
Fen.panc.
D. chol.
Panc.dors.
Fen.chol.
D.p.v.
Pars inf.

9


Cav. dext.
Cav. sin.
D.p.d.
D.h.-p.
Cav. dext.
Cav.sin.
Vac.

10


T.musc.
Panc.dors.
D.chol.
D.h.-p.
Panc. vent.
T.muc.
Pars inf.
Tau Brodh '35

11

PLATE 2

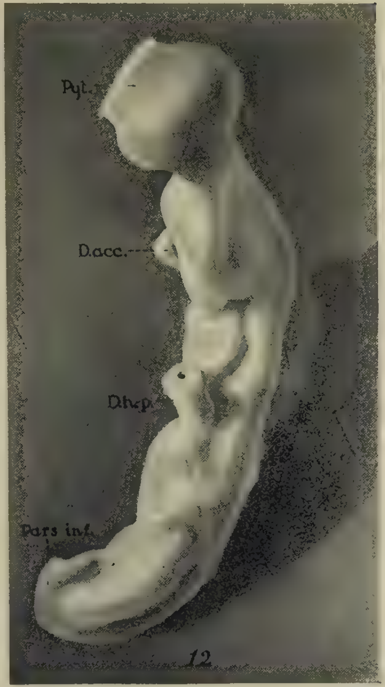
EXPLANATION OF FIGURES

Wax models of pars descendens duodeni of an 18-mm. human embryo (H-260), illustrating reestablishment of lumen through coalescence of vacuoles.

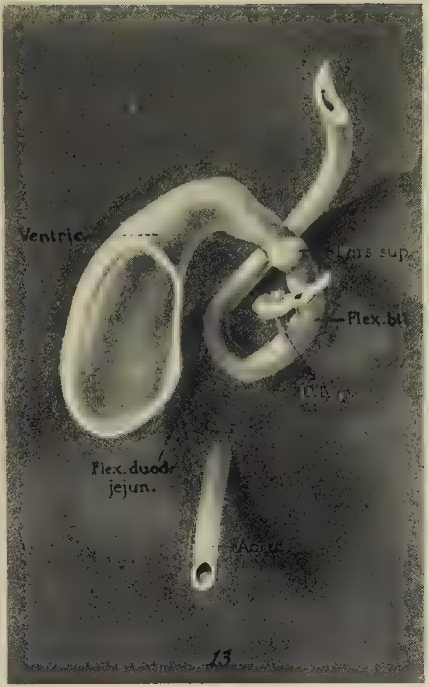
12 Epithelial anlage of pars descendens, viewed from posterior side (same orientation as in fig. 13). Note that the two channels shown in figure 14 are represented by surface bulges in figure 12. *D. acc.*, accessory pancreatic duct (duct of dorsal pancreas); *D.h.-p.*, hepato-pancreatic duct (primitive ampulla); *Pyl.*, pylorus; *Pars inf.*, inferior portion of duodenum. Magnification × 90.

13 Epithelial primordium of stomach and small intestine, shown at smaller magnification (× 22.5), for purposes of orientation. *Flex. bil.*, biliary flexure of pars descendens of duodenum. In all embryos studied, the orifice of the ampulla lies just below or just above this bend, hence the name, biliary flexure.

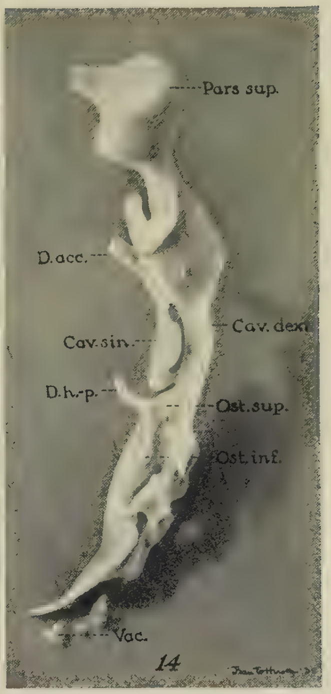
14 Wax cast of lumen of duodenum illustrating relation of orifices of ampulla to right and left channels (*Cav. dext.*, *Cav. sin.*). *Ost. sup.*, *Ost. inf.*, upper and lower orifices of ampulla; *Vac.*, vacuoles which have not yet coalesced. (In general, coalescence takes place in a cephalo-caudal direction). Magnification × 90.


Pyl.
D.acc.
D.h.p.
Pars inf.

12


Ventric.
Pars sup.
Flex. bil
D.h.p.
Flex. duod. jejun.
Aorta

13


Pars sup.
D.acc.
Cav. dext
Cav. sin.
D.h.-p.
Ost. sup.
Ost. inf.
Vac.

14

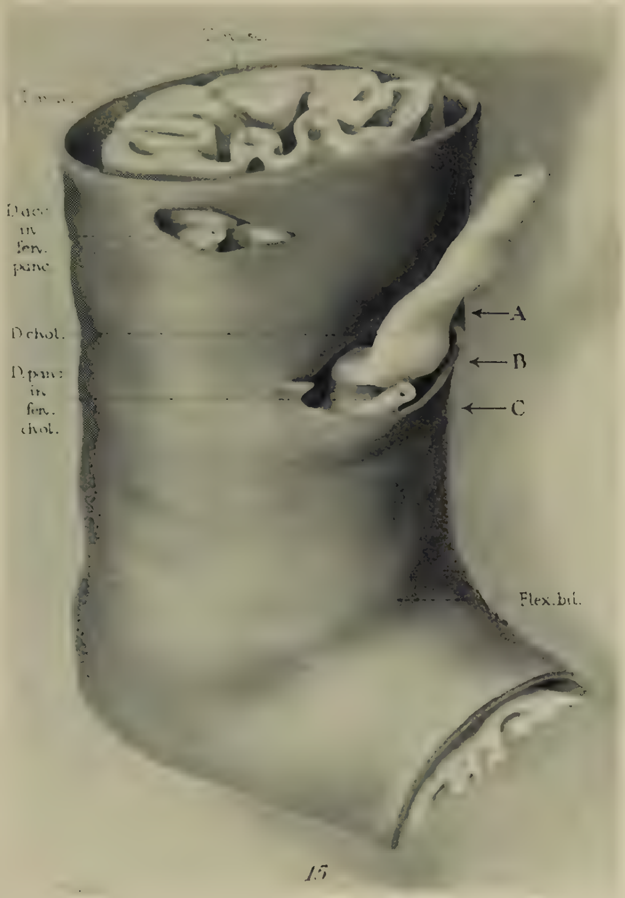
PLATE 3

EXPLANATION OF FIGURES

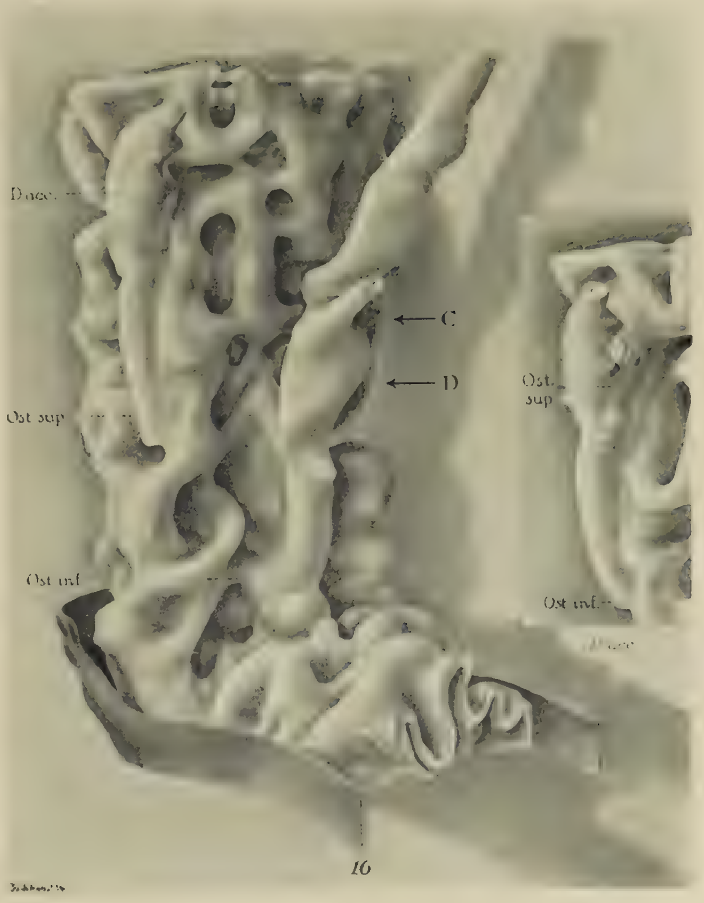
Wax model of pars descendens duodeni of a 33-mm. human embryo (H-16). Magnification × 70.

15 Pars descendens viewed from left side to show relation of ducts to 'windows' in tunica muscularis. Note that upper margin of the fenestra choledocha is pressed inward and that lower margin bulges. This window will have the same shape in the next older fetus. (See plate 2 of next article). *Flex. bil.*, biliary flexure of duodenum. For transverse sections, at levels *B* to *D*, see figures 17 to 19.

16 Pars descendens stripped of muscle layer to show submucosal surface of mucosa and position of double orifice of ampulla. (The insert presents a more lateral view of the accessory pancreatic duct.) Same orientation as in figure 15. *D. acc.*, accessory pancreatic duct; *Ost. sup.* and *inf.*, upper and lower ostia of ducts.


D.acc. in ferv. panc.
D.chol.
D.panc. in ferv. chol.
A
B
C
Flex.bil.

15


D.acc.
Ost sup
Ost inf
C
D
Ost. sup
Ost inf.

16

PLATE 4

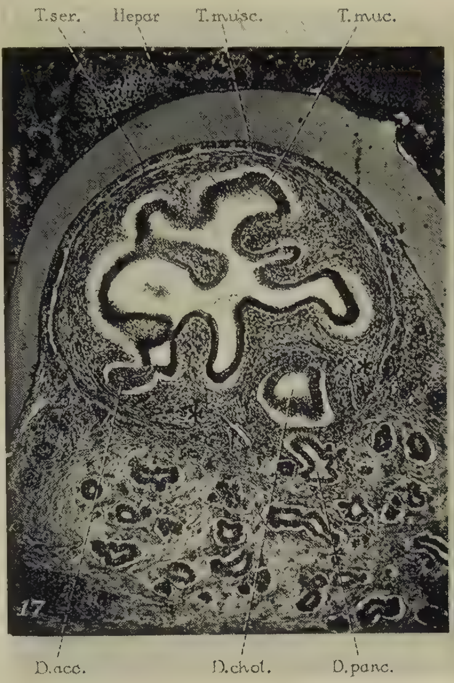
EXPLANATION OF FIGURES

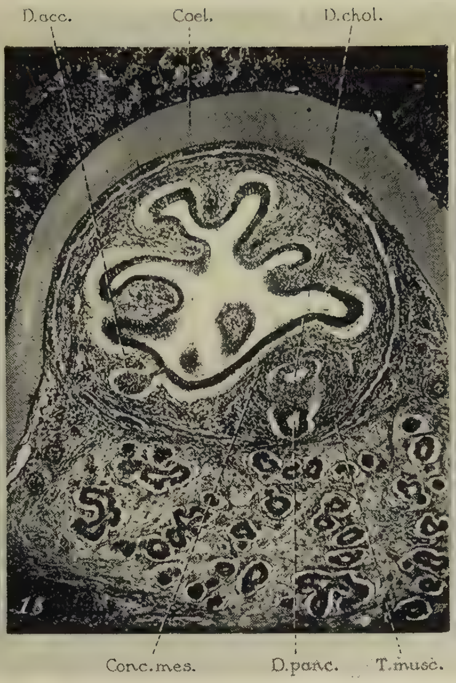
Three transverse sections through the duodenum of the 33-mm. human embryo shown in plate 3. Magnification, × 60.

17 Level *B,* of figures 15 and 16, the level at which the bile and pancreatic ducts (*D. chol. and D.-pan.*) enter the hiatus in the tunica muscularis of the duodenum. The asterisks indicate the two lateral margins of the 'window' (*fen. chol.*). Note the concentric arrangement of the mesenchyma about the common bile duct as it lies in the submucosa. This is the first suggestion of an intrinsic musculature. *T. ser., T. musc., T. muc.,* three tunics of the duodenum.

18 Level *C,* of plate 3, illustrating the junction of bile and pancreatic ducts within the tunica submucosa of the duodenum. Again, note concentric arrangement of mesenchyme (*conc. mes.*) about the two ducts.

19 Level *D,* of figure 16, showing primitive ampulla (*D. h.-p.*) and its upper orifice (*Ost. sup.*). Note difference in staining reaction of duct and epithelium of duodenal mucosa. *Panc.,* pancreatic alveoli.


T.ser.
Hepar
T.musc.
T.muc.
17
D.acc.
D.chol.
D.panc.


D.acc.
Coel.
D.chol.
18
Conc.mes.
D.panc.
T.musc.

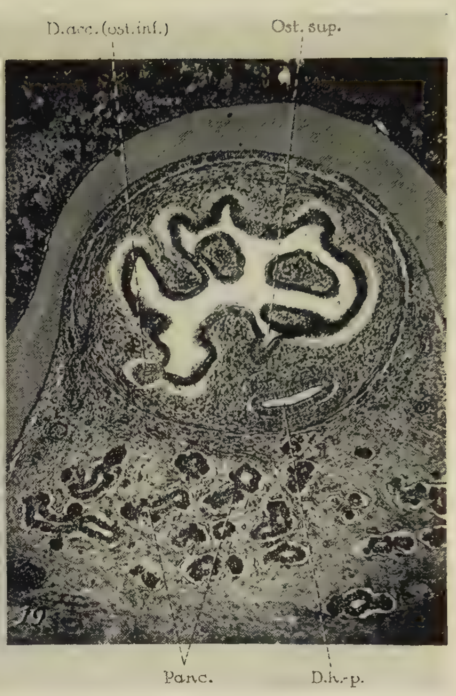

D.acc.(ost.inf.)
Ost.sup.
19
Panc.
D.h.-p.

PLATE 5

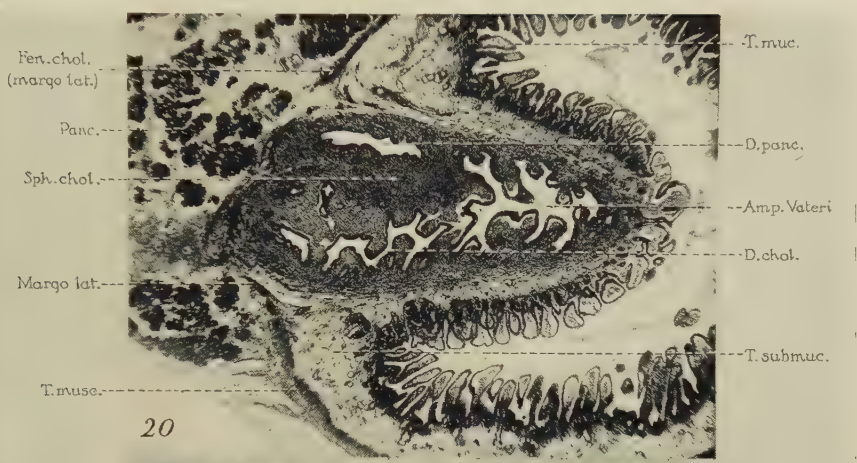
EXPLANATION OF FIGURES

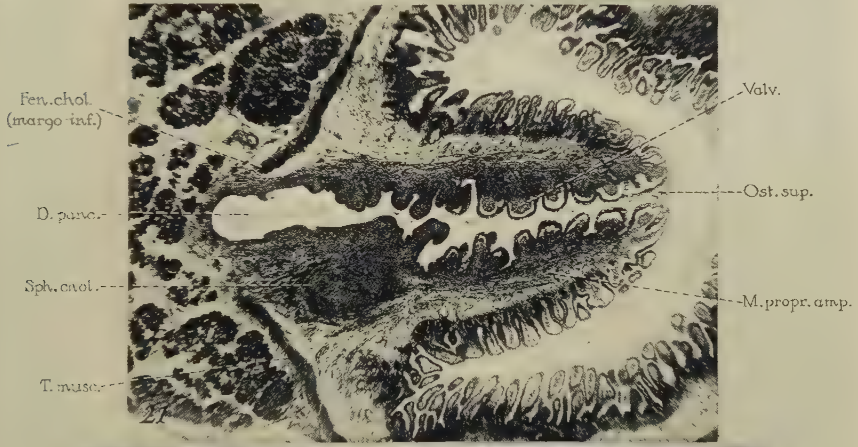
Transverse sections of the pars intestinalis of the 36.5-cm. fetus shown in figure 6. Magnification, ×17. These sections illustrate the persistence of two orifices of the ampulla.

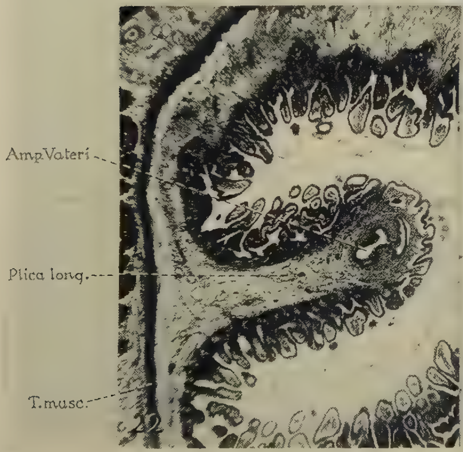
20 Level *B* of figure 6. Note lateral margins of choledochal fenestra, the junction of bile and pancreatic ducts to form the ampulla of Vater and the presence of a musculus proprius around and between the two ducts, especially the sphincter choledochus (*Sph. chol.*), to be discussed in the next article.

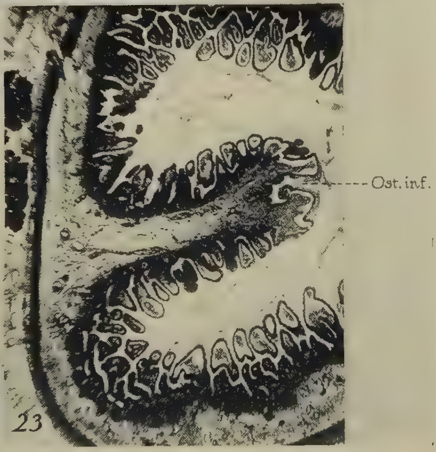
21 Level *C* of figure 6. Level of upper ostium and level at which the pancreatic duct enters the intestinal window. The section is taken just below the bile duct, but the sphincter fibers of the duct are still visible, cut tangentially (*Sph. chol.*), Note longitudinal fibers (*M. propr. amp.*) and valvules of ampulla (*Valv.*).

22 and 23 Levels *E* and *F* of figure 6, just above and through the inferior orifice of the ampulla. This portion of the plica longitudinalis would probably have developed into a frenulum.


20
Fen.chol. (margo lat.)
Panc.
Sph.chol.
Margo lat.
T.musc.
T.muc.
D.panc.
Amp.Vateri
D.chol.
T.submuc.


21
Fen.chol. (margo inf.)
D.panc.
Sph.chol.
T.musc.
Valv.
Ost.sup.
M.propr.amp.


22
Amp.Vateri
Plica long.
T.musc.


23
Ost.inf.

A TECHNIQUE FOR STUDYING THE INNERVATION OF ORGANS

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Department of Gynecology, Johns Hopkins University and Department of Embryology, Carnegie Institute of Washington

We are publishing this technique because it has been extremely helpful to us in the past 6 years. We have used it in dissecting the nerve supply of the ureter, kidney and bladder of the human being, and the reproductive organs of the female monkey, Macacus rhesus. It is particularly adapted to the study of fine plexuses and ganglia, the innervation of blood vessels and pedicles and the dissection of nerves in their relation to organs.

The principle which underlies this method is that the tissue is fixed and stained permanently before it is dissected. After it has been fixed and stained, moreover, the tissue remains soft and pliable, so that it can be dissected easily. By this technique, many other tissues also are stained, such as muscle, blood vessels and connective tissue, but since nerve takes the stain most deeply, the contrast is striking and clear.

We evolved this technique 6 years ago while we were searching for a satisfactory method of dissecting the nerves of the human ureter. We started our investigation of the ureteral innervation in the usual way, making gross dissections of cadavers, either fresh or fixed by formalin. After 6 months, however, we realized that such dissections would not answer our needs. In the first place, they did not reveal fine and delicate structures. Even when using optical lenses, we could not analyse plexuses, ganglia and nerve pathways as accurately as we wished to. Then, again, it was impossible to identify the nature of finer structures, and distinguish nerves from blood vessels even with a magnification of 5 or 6 diameters. Of course, one could always excise a piece and stain it; that, however, ruined the dissection both for purposes of demonstration and photography. Then, too, the illustration of such dissections became a problem. Ordinary dissections rarely lend themselves to photography, and drawings, although beautiful, may be inaccurate and are always expensive.

In reaching these conclusions, we did not underestimate the value of gross anatomical dissections. They are of indispensable value for they give one a general view of anatomical relationships, enable one to plan further particular study and teach one how to approach a given region surgically. But the adequate study of the innervation of an organ requires a greater detail than that.

The technique which we describe is apparently unique in that it preserves the gross specimen in its normal relationships and yet enables one to dissect and study it microscopically. It also enables one to dissect a given region at leisure, because the tissue has been permanently fixed; the tissue is soft, clean, pliable and has no odor; we can recognize structures as we come to them, because they are stained; and when we are through, we have a dissected specimen which we can keep, photograph, mount or later redissect, as we wish.

For the resurrection of the formulae of the fixing and staining fluids, we are indebted to Mr. Charles Miller, for many years a technician in the department of anatomy under the late Dr. Franklin P. Mall and more recently in the department of embryology of the Carnegie Institute. Mr. Miller found the formulae on some old cards in his index file, and gives the credit for these formulae to the late Doctor Sihler, who evolved them many years ago while in the department of biology in the Johns Hopkins University. Dr. Sihler apparently gave the formulae to Doctor Mall, who in turn gave them to Mr. Miller. Had it not been for Mr. Miller's systematic methods, they would have been lost.

We have made a careful search but can find no indication that Doctor Sihler ever published the formulae of these fluids.

In 1900, in a study entitled, "Die Muskelspindeln, Kerne and Lage der motorischen Nervenendigungen," he made no reference to this method of staining. The only reference to it that we can find in the literature is in a book entitled "Physiological Histology. Methods and Theory," by Gustav Mann, of Oxford University, 1902, and we cannot be certain that Mann refers to Sihler of Baltimore because the name is spelled differently (Siehler and also Seihler). Also Mann gives the formula of only the first fluid and his prescription required only one-third as much acetic acid as Sihler used. No mention is made of the other fluids, particularly the staining technique, which are indispensable parts of the process. In their original form they came into the possession of Mr. Charles Miller before 1900.

During the last 6 years, we have modified these formulae, have omitted one of the solutions used and have simplified the technique of staining. We have also found out how to handle tissues that have been stained too deeply or too lightly. We have also learned how to make permanent and convenient preparations of these dissections by mounting them on glass in Canada balsam. We have also discovered the particular types of dissection which have been adaptable to this method of study. All these features we have combined into a technique which would have been impossible without the methods of fixation and staining; for these we are indebted to Doctor Sihler and Mr. Charles Miller.

TECHNIQUE

1. The tissue

The tissue must be moderately fresh, 24 or 48 hours old, and should not have been fixed or treated in any way. We like to get the tissue as fresh as possible. The region to be studied is removed in one piece, a total mount. Thus, if one wishes to study the innervation of a solid organ like the ovary, uterus or kidney, the organ is dissected out with the surrounding tissue in which the nerves lie. This whole dissection is then stretched out flat on a piece of good writing paper and allowed to adhere to it. This will take about 5 or 10 minutes. The whole preparation, including the paper, is then placed into solution A. The tissue often adheres to the paper until the final immersion in glycerin; this keeps the dissection flat and thin, is desirable but not necessary.

In excising the area which we wish to stain and dissect, certain precautions may be observed. The tissue should be as thin a sheet as possible. This facilitates later study, for a thin sheet of tissue is much easier to handle and much more quickly dissected than a thick one. We have found this method unsatisfactory in dissecting a solid organ like the kidney, ovary or uterus, whereas it is ideal for studying nerve paths that lie in a fairly thin plane, such as the ovarian or renal pedicle, the various abdominal and pelvic plexuses and the branches that pass from those plexuses to various viscera. Hollow viscera like the ureter and bladder can be stretched out and dissected very clearly.

In general, since it is helpful to have thin dissections, we have done most of our work on human infants and adult monkeys.

It is advisable to make a careful preliminary gross dissection of the particular region one wishes to investigate in order that one may be able to eliminate unnecessary tissue in the final dissection. When a wide area or several planes of tissue are to be studied, it may be necessary to prepare several specimens for fixing and staining. Each dissection is something like a microscopic section, except that it is much thicker and enables one to follow a given structure as far as the dissection goes.

2. *Solution A*

Pure glycerin	1 part by fluid measure
Glacial acetic acid	1 part by fluid measure
1% aqueous chloral hydrate solution	6 parts by fluid measure

The tissue is allowed to remain in solution A for 18 hours and then transferred directly to solution B.

3. Solution B

Pure glycerin	1 part by fluid measure
Ehrlich's hematoxylin	1 part by fluid measure
1% aqueous chloral hydrate solution	6 parts by fluid measure

It is allowed to remain in solution B for 24 hours or longer, until it is properly stained, when it becomes a deep purplish blue. If there is any doubt, a small bit of tissue can be cut off and examined under the microscope, to see whether it is sufficiently stained.

After it has been stained to the proper degree, the tissue is then placed in pure glycerin where it can remain indefinitely. It becomes deeper blue after it is put into glycerin. It is necessary to change the glycerin after 10 days or so, because of the acid in the tissue. The color of the tissue turns deeper blue as it gets more alkaline.

If overstained, it should be discolorized to the proper point in acid alcohol (alcohol 70%, hydrochloric acid 1%), or by putting it back into solution A. The tissue is then neutralized by washing it in distilled water to which have been added a few drops of saturated aqueous solution of lithium carbonate or by placing it in pure glycerin. In a few days, it will be ready for dissection. It can remain indefinitely in glycerin.

A specimen that is weakly stained can always be transferred from glycerin to solution B for restaining, thus making it possible to save valuable dissections. This emphasizes the flexibility of this technique.

4. Preparation for dissection

The tissue is kept in glycerin constantly, and the dissection is made in this fluid. It is necessary to stretch out the tissue tightly and fix it so that it may be studied. To do this we have used flat bottomed, round glass dishes, like deep Petri dishes, which have flat covers. These dishes can be obtained in various sizes, depending upon the size of the dissection. Into the dish is poured enough melted paraffin to make a layer about 1 cm. thick. After this has hardened, it forms a firm, translucent base on which the dissection can be pinned. This base also transmits light, so that by reflectors, a beam can be thrown up through the tissue for dissection or photography. We also use overhead light particularly when dissecting thick specimens. We have discarded substage lamps because their heat melts the paraffin.

The dissection is done by aid of the microscope; we use a binocular, with a magnification of from 5 to 25 diameters. The dissecting instruments must be fine and probes and forceps must have sharp points.

5. *The mounting of the specimen*

If the specimen is not too thick, it can be mounted very readily in Canada balsam. We first tried to mount our specimens in glycerin, but gave this up as the expansion and contraction of the glycerin caused the mounts to leak. If one wishes to mount the specimen in Canada balsam, one can remove the glycerin, dehydrate and clear in the following manner.

1. Run tissue through alcohol (70, 80, 95%) to remove glycerin.
2. Clear in solution consisting of

Bergamot oil	2 parts
Oil of cedar	2 parts
Pure carbolic acid crystals, liquefied by heat	1 part

3. Xylol
4. Mount in Canada balsam

We have also found that small preparations can be dehydrated for mounting in Canada balsam merely by washing the preparation in several changes of dioxan. We have not yet tried this technique on large dissections.

REMARKS

By this technique, almost all tissues are stained, muscle, connective tissue, blood vessels; nerve, however, is stained particularly well. This method has none of the idiosyncracies of the gold and silver stains and is completely dependable. The stain does not fade. Mr. Miller has demonstrated nerve endings in voluntary muscle by this technique; we have not tried, however, to demonstrate them in any of our dissections. If there are any who would like to see photographs of such dissections, they might refer to the Journal of Urology, vol. XXVIII, no. 6, December, 1932, where some of our photographs have been printed, or to the next issue of this journal in which we publish some photographs of dissections made with this technique.

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SIHLER, CHRIS 1900 Die Muskelspindeln, Kerne und Lage der Motorischen Nervenendigungen. Arch. f. Mikros. Anat. und Entwick., Bd. 56, S. 334–354.

THE HISTOLOGY OF THE MAMMARY GLANDS OF ADRENALECTOMIZED LACTATING RATS[1]

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TWO PLATES (FOURTEEN FIGURES)

The hormones at present recognized to control the mammary gland as regards duct growth, lobulation and finally its secretory activity come principally from the ovary and the anterior lobe of the pituitary. It has been shown experimentally that the thyroids, uterus, placenta, and the adrenal cortex also play a part, as yet not completely determined, in the initiation and the continuance of secretion. These relationships have been adequately reviewed by Nelson ('36), Carr ('31), Gaunt ('33), Brownell et al. ('33), Selye et al. ('34). Britton and Kline ('35) and Gaunt and Tobin ('36) have shown that after adrenalectomy in rats and cats lactation fails or is impaired. These deficiencies can be remedied, according to Brownell et al., only by an adrenal hormone, distinct from the life maintaining cortical hormone; and according to Gaunt and Tobin they can be alleviated by large dosage of the cortical hormone. Nelson and Gaunt ('36) have shown that, in guinea pigs at least, the adrenal cortex is an important, and probably necessary, factor in the initiation of lactation.

The purpose of this paper is to describe the histological changes in the mammary gland of the rat accompanying mammary dysfunction following adrenalectomy and to see if such observations will contribute to an analysis of the nature of that dysfunction.

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[1] Accepted in partial fulfillment of the requirements for the degree of Master of Science at New York University.

Grateful acknowledgment is hereby given to Dr. Harry A. Charipper and to Dr. Robert Gaunt for constructive criticism and stimulus for carrying on this work.

MATERIAL AND METHODS

Control preparations were made from small pieces of mammary gland removed from two normal animals beginning from the fourteenth day of pregnancy and continuing every 2 or 3 days throughout the remaining gestation period, the whole of the lactating period, and during the first week after the young had been weaned. Care was taken to remove only small sections of gland so as not to reduce materially the amount of lactation directly due to reduction in glandular tissue. No consecutive biopsy was made on the same gland. Healing was quite rapid and the nipple showed no damage. This procedure offered histological material from the mammary gland which could be used for relating the structure of the tissue with the amount of milk being secreted. The rate of secretion was judged by the weight of the litters. To have a uniform basis of comparison all the litters used were reduced to four in number. Daily weight records were kept of the mothers as well as of their litters and the growth curves of the litters corresponded closely to those of unoperated animals showing that the operative technique did not in any way hinder normal development of the young.

Next ten rats were adrenalectomized on the first day after delivery and samples of their mammary glands removed at biopsy every 2 or 3 days thereafter as in the control experiment. A day after parturition secretion of the mammary gland usually becomes abundant, and any subsequent change from normal can be attributed to adrenalectomy.

Another group of seven animals were adrenalectomized 1 day after delivery and treated in the same way as the above experimental group except that they were given a 0.9% sodium chloride solution in place of plain water to drink.

Two animals were allowed to litter normally. When their litters were several days old and appeared to be growing normally the mothers were deprived of water for 4 days.

Samples of mammary tissue were removed at the beginning and at the end of the water deprivation period.

The glands were fixed in Bouin's fixative and cleared in cedar oil. After embedding, sections from 6 to 8 μ in thickness were cut and the slides stained with Delafield's haematoxylin and counterstained with eosin. Some of the sections were run through Mallory's triple connective tissue stain and Weigert's elastic stain so that the change in the amount of elastic fibers and quantity of connective tissue elements could be studied.

TABLE 1

Growth of the litters of untreated adrenalectomized mothers

LITTER NO.	AVERAGE WEIGHT OF SUCKLING YOUNG					
	On 1st day	On 4th day	On 8th day	On 12th day	On 16th day	On 20th day
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
R. 6[1]	6.0	7.5	9.7	11.2	11.5	10.5
R. 8[1]	5.6	7.4	10.0	12.7	16.0	15.0
R. 3	6.8	9.2	11.0	12.2	11.5	Died on 17th day
R. 4	5.5	7.4	10.2	12.0	9.3	Died on 16th day
R. 2	5.1	8.7	11.5	11.5	Died on 14th day	
R. 9	5.6	8.2	9.9	9.0	Died on 14th day	
R.10	5.0	5.1	6.0	6.5	Died on 14th day	
R. 5	5.6	6.2	6.0	4.0	Died on 13th day	
R. 1	7.0	7.5	8.0		Died on 10th day	
R. 7	5.6	7.3	8.2		Died on 10th day	
Normal[2]	6.0	8.1	13.8	19.2	25.1	33.4

[1] Litters survived, macroscopic cortical tissue found on renal veins in both animals.

[2] Average weights of eight litters taken from unoperated animals.

EXPERIMENTAL OBSERVATIONS

A. General

In the present work, all bilaterally adrenalectomized untreated animals, excepting two which showed macroscopic adrenal accessories, were unable to rear their young, the litters living 9 days or longer under the experimental conditions employed here. A comparison of the weights of these litters with the weights of normal litters showed that the growth rate of the experimental young was greatly retarded (table 1). The average weight of the young which lived for

10 days after adrenalectomy of the mother was 5 gm. below the normal weight for this laboratory. In those litters living 15 days the normal young were 12 to 15 gm. heavier than the experimental young. The two mothers, shown at autopsy to have macroscopic accessory cortical tissue, lactated just enough to raise their litters. At 20 days of age, one of the litters had an average weight of 10.5 gm. and the other 15.0 gm. which is less than half the normal weight. In all cases normal maternal care was attempted. During the period that the litters were alive the mothers showed no marked signs of adrenal insufficiency, losing an average of 11.3 gm. per animal.

TABLE 2

Growth of the litters of salt treated adrenalectomized mothers

LITTER NO.	AVERAGE WEIGHT OF SUCKLING YOUNG					
	On 1st day	On 5th day	On 10th day	On 15th day	On 20th day	On 30th day
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
R.11	5.9	9.0	12.7	14.5	20.0	57.0
R.12	5.0	8.2	12.7	21.0	28.0	63.3
R.13	7.0	9.2	11.2	13.2	14.0	29.0
R.14	5.6	9.5	12.0	12.2	14.0	30.0
R.15	5.3	9.0	13.0	15.5	21.5	52.6
R.16	5.0	7.7	7.0	Died on 11th day		
R.17	5.0	5.3		Died on 6th day		
Normal	6.0	10.0	16.1	23.0	33.4	63.6

Of the mothers that were given saline solution to drink, five survived and two died, one on the fifth and one on the tenth day after the birth of the litter. The litters of the five surviving mothers showed, in three cases, weights very close to normal and, in two cases, about half the normal (table 2).

B. Histology of the mammary glands of untreated adrenalectomized animals correlated with rate of secretion

1. Mammary glands shortly after adrenalectomy. In a portion of mammary gland removed at biopsy from lactating mothers 1 or 2 days after adrenalectomy, the parenchyma was more prominent than the connective tissue. The glandular tissue was loosely arranged and the alveolar cells were in various stages of active secretion. Milk was present in the ducts and the lumina of the gland but the alveoli did not reach the distention found in the normal animal at this time (fig. 1). Even in the fullest lumen there was seldom enough secretion present to cause a flattening out of the epithelial cell nuclei. The secretion found in the ducts and the acini appeared to consist mainly of solid material. It stained deeply and was only slightly vacuolated. For the most part the acinar cells were cuboidal or low columnar, somewhat vacuolated, with a small granular nucleus which stained darker than the cell nucleus of a normal gland. The nuclei of these cells were smaller than those found in the cells of normal glands removed at the same period of lactation. The chromatin material was arranged around the periphery of the nucleus and a well-defined nucleolus, centrally placed, was present. In secreting cells the nucleus was found at the basal end of the cell, the apical portion of the cell facing the lumen being filled with a large fat droplet.

2. Mammary gland at the height of secretory activity. Pieces of gland removed from the same animals, described in (1) above, when the litters had reached their maximum weights, had a general appearance similar to the one described above. Sections showed many fairly well-filled alveoli (figs. 2 and 3) with but little adipose tissue present. The alveoli, however, were still not maximally filled or distended. The cells, lining the lumina which have been emptied, were columnar in shape and contain secretory products. The cell nuclei stained darkly with an occasional pycnosis apparent. The milk found in the ducts and the acini took up more stain than that found in a normal gland in the same period of lactation. This seemed to be due to a more compact nature of the milk found in the adrenalectomized animal. Samples of gland removed at any time while the young were either gaining or remaining the same in weight showed essentially the same picture.

3. Mammary glands after the death of the litter. A section of mammary gland (fig. 5) removed from the same animals, described in (1) and (2) above, after their young had died differed from the above only in that there was an increase in connective tissue so that now glandular tissue and stroma were in equal proportions. The stroma consisted mainly of connective tissue septa which surrounded the lobules. Adipose tissue was found in all parts of the section filling up the space between the ducts and the alveoli. The lobules consisted of many closely packed alveoli containing a little milk in their lumina. This milk stained as a solid mass with little vacuolation present. Secretory activity was going on in some of the acinar cells. Here, in contrast to the cell nucleus of a normal animal lactating for the same period of time, the nucleus was granular instead of vesicular. Milk, even after the litter had died, was found in the lumina and ducts of the gland. This shows that adrenalectomy, unlike the reputed effects of hypophysectomy, did not stop lactation completely but only reduced its level to such an extent that the litters generally died.

C. The histology of the mammary glands of salt treated adrenalectomized animals

1. Mammary gland during the lactating period. Sections of mammary gland (figs. 6, 7 and 8) removed at any time from animals which, due to the salt treatment, were able to rear their young resembled more closely in histological structure the glands of normal lactating animals than the glands of adrenalectomized untreated animals. The glands consisted of closely packed, distended alveoli with but little connective tissue separating them. The alveoli were filled with a vacuolated milk which stained in a manner similar to that of an untreated operated animal. The lining acinar cells ranged from low columnar to long flattened out cells with indistinguishable boundaries. The nuclei of these cells were as small as those found in the glands of untreated operated animals. They were granular in appearance as opposed to a large clear staining vesiculated nucleus found in a normal lactating gland. The effect, in general, of salt treatment, as here given, is to repair largely, but not entirely, mammary structure and function after adrenalectomy.

D. The histology of the mammary glands of normal lactating mothers deprived of water

Water deprivation caused a decrease in the secretory activity of the mother as is evidenced by a loss in the weights of the litters. Sections of gland removed just before deprivation of water were normal in appearance (fig. 13). Large lumina were present containing a light staining vacuolated milk. When the mother had been without water for 4 days the appearance of the mammary gland changed (fig. 14). The lumina were still distended with milk but now their contents stained darkly and appeared of a more solid consistency. The milk was similar in appearance, although somewhat greater in amount, to that found in the mammary glands of untreated adrenalectomized animals.

DISCUSSION

That deficiencies occur in the lactation of adrenalectomized animals has been well established and is verified here. There have been two explanations offered as to the cause of and remedy for these deficiencies. Brownell et al. ('33) attribute them to the lack of a cortical hormone, which they designated as cortilactin. Gaunt and Tobin ('36) suggested that the deficiencies were due to the well-known disturbance in fluid balance associated with adrenal insufficiency, rather than to the lack of any specific adrenal lactogenic factor. They found that the deficiencies could be largely or completely remedied either by small doses of cortical hormone plus salt treatment or by large dosage of cortical hormone alone.

The present observations do not offer conclusive proof as to the validity of either of these two explanations but a study of the histological structure of the glands of untreated adrenalectomized animals shows that the changes found are not of such magnitude as to suggest the loss of a hormone specifically concerned with maintaining mammary function. The secretory elements of the mammary gland such as the secretory epithelial cells and lumina are present as can be seen by examination of figures 1 to 5. The acinar cells are found in the stages of milk secretion as described by Weatherford ('29). However, these secretory elements do not function at a normal rate. This is indicated by the non-distended alveoli found in the glands of untreated adrenalectomized animals. This difference in distention between the glands of operated and normal animals is always found. As secretion can be judged by the degree of distention of the alveoli, which is dependent upon the contained milk, there must be a subnormal milk formation going on in the glands of adrenalectomized animals.

Not only do adrenalectomized animals secrete less milk but the milk that they do secrete is not normal in appearance. This is indicated by different staining properties and apparently a markedly greater milk density. Milk found in sections of gland removed from untreated adrenalectomized animals appears very compact and stains more heavily than does the milk of normal glands. It can be reasoned from this that less fluid is in the milk of the untreated operated animals.

These observations could all be interpreted as indicating that the deficiency is directly due to the lack of fluid for milk secretion. It has been recognized by many workers (Rogoff and Stewart, '26; Swingle et al., '33; Harrop and Weinstein, '33, and others) that after adrenalectomy there is a loss in haemal fluids. This hemoconcentration would further lend strength to the assumption that the deficient factor of milk secretion is fluid. An additional substantiation may be found in the fact that treatment with saline solutions will largely remedy the lactation deficiency following adrenalectomy, whereas, deprivation of fluids in the normal animal tends to simulate the decrease in mammary gland function found experimentally.

Secretory activity does not come to a halt immediately after adrenalectomy but is slowly decreased. If reduced secretion was brought about by the removal of a lactogenic hormone cessation of milk would probably occur much sooner than it did in this experiment (Selye et al., '34 and Nelson, '35).

The fact that milk secretion only gradually slows down after adrenalectomy and does not completely stop until the stimulus of suckling is removed suggests again that the deficiencies are due to the general metabolic condition of the animal, most likely centering about its water balance.

CONCLUSIONS

1. The mammary glands of untreated adrenalectomized rats which do not secrete at a normal rate show only slight structural changes from normal.
2. Secretory elements are present and functional. The acinar cells undergo vacuolization and throw their secretion into the lumina. The most striking difference is that the alveoli are less distended and apparently contain a more dense secretion.
3. Although the litters of untreated adrenalectomized rats generally die of starvation, milk secretion does not completely stop as long as the litters are still suckling.
4. Salt therapy will, in most cases, largely restore the normal histological picture of the mammary glands as well as their function.
5. Water deprivation produces changes in the secretory content of mammary glands similar to those found in untreated adrenalectomized animals.
6. These results are interpreted as a substantiation of the theory that the deficiencies following adrenalectomy are due largely to the disturbance in water and salt balance.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

All figures are taken at the same magnification, × 240.

1 Mammary gland (R.1) removed on the second day of lactation. Note the slightly distended alveoli and their scantily filled compact contents. Compare with figure 9.

2 Mammary gland (R.1) removed on the fifth day of lactation. Note comparative emptiness of alveolar lumina and the height of the acinar epithelial cells. Compare with figure 10.

3 Mammary gland (R.1) removed on the eighth day of lactation. Lumina are small and filled with a compact secretion. Note the amount of connective tissue between alveoli. Compare with figure 11.

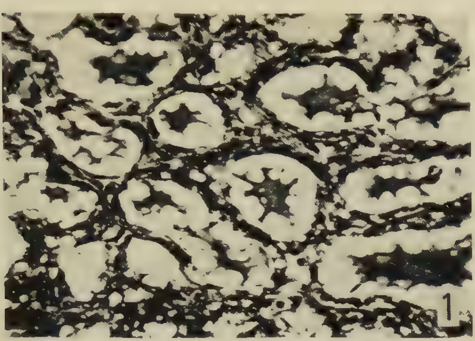
4 Mammary gland (R.1) removed on the ninth day of lactation. Note increase in connective tissue and compactness of whole gland. The alveolar secretion stains darkly and appears more solid.

5 Mammary gland (R.1) removed on the tenth day of lactation and directly after the litter had died. Stroma and connective tissue are present in equal proportion. Some milk is still found in the few remaining alveoli.

6 Mammary gland (R.12) on salt therapy removed on the fourth day of lactation. The lumina are distended and filled with secretion. Compare with figure 10.

7 Mammary gland (R.12) on salt therapy removed on the tenth day of lactation.

8 Mammary gland (R.12) on salt therapy removed on the sixteenth day of lactation.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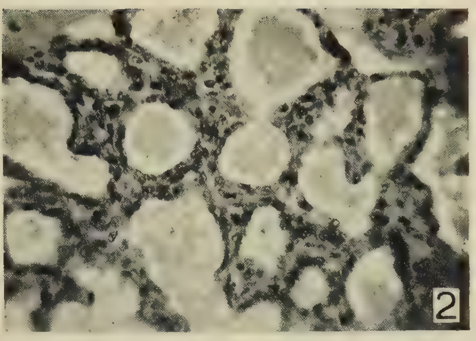
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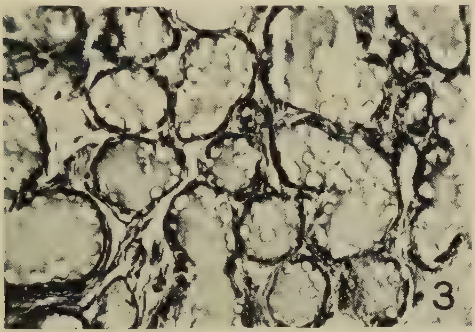
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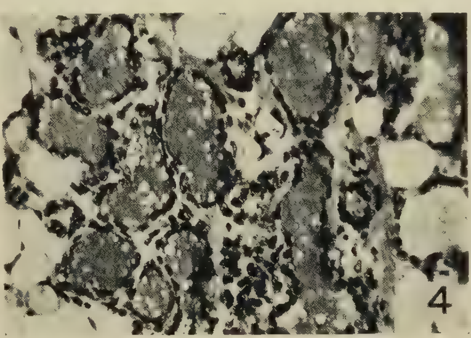
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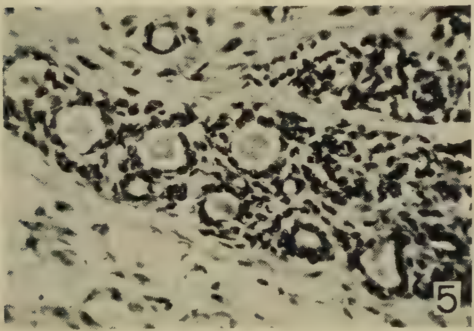
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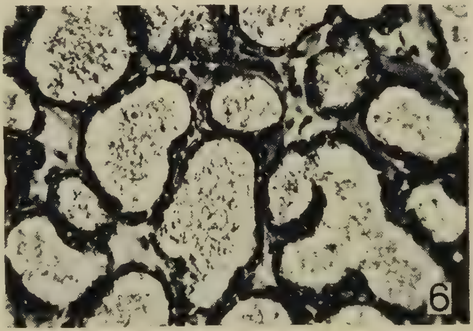
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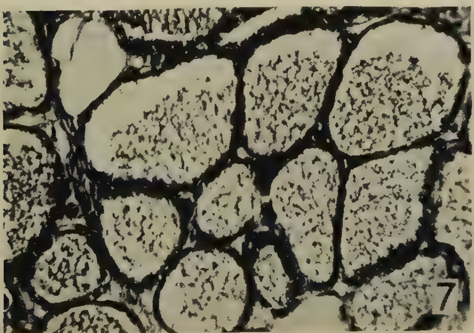
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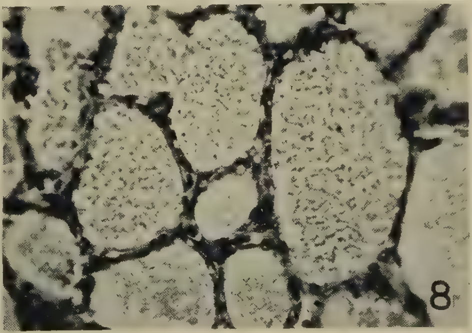
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PLATE 2

EXPLANATION OF FIGURES

9 Mammary gland removed from a normal animal on the second day of lactation. Note the distended alveoli, the vacuolization of their secretory contents and its light staining reaction.

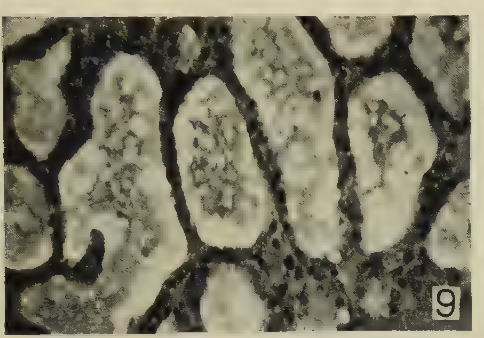
10 Mammary gland removed from a normal animal on the fifth day of lactation. Note comparative fullness of alveolar lumina and flattened condition of acinar epithelium. Compare with figure 2. Also note the similarity to figure 6.

11 Mammary gland removed from a normal animal on the eighth day of lactation.

12 Mammary gland removed from a normal animal on the fifteenth day of laction. Compare with figure 8.

13 Mammary gland removed from a normal lactating animal just before being deprived of water. Note the vacuolated and lightly staining alveolar contents.

14 Mammary gland removed from the same animal after being deprived of water for 4 days. The alveolar contents are now more compact and take up more stain. Compare with figures 2, 3 and 4 and note difference as compared to figure 13.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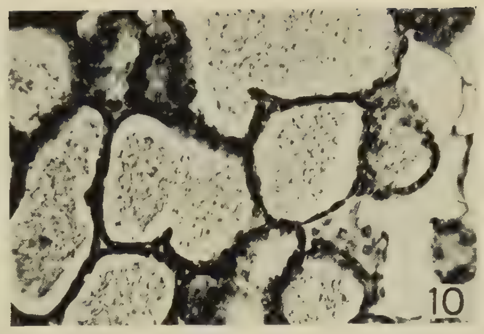
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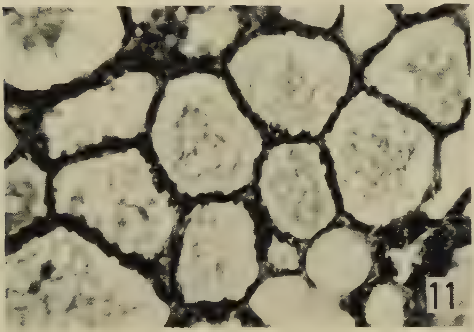
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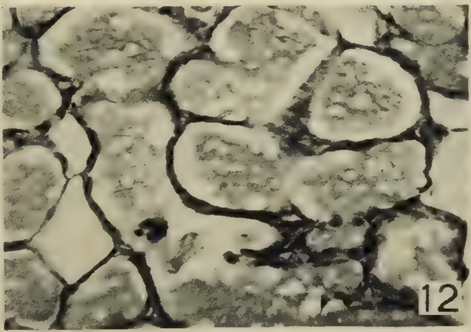
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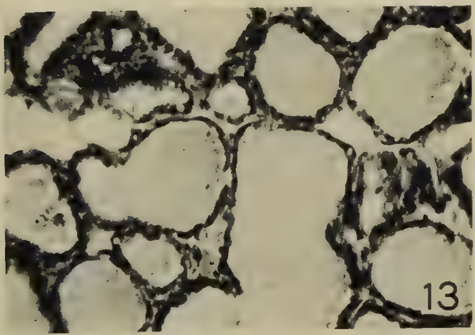
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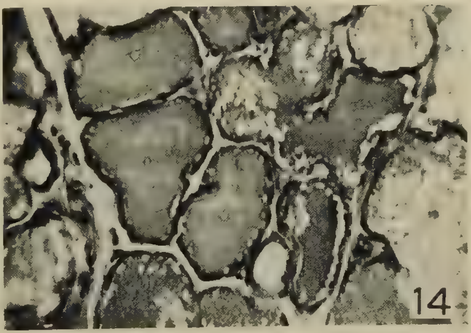
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AN INEXPENSIVE CASTING MATERIAL FOR MAKING ANATOMICAL REPRODUCTIONS

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ONE PLATE (FIVE FIGURES)

In our studies of growth and development accurate plastic reproductions of dissections of fetuses and infants were desired in order to have three-dimensional records in addition to drawings and photographs. Photographs and drawings serve their purpose well enough as records but have not many of the advantages of accurate, permanent casts which are true reproductions made directly from dissections by means of negative impressions. Since the cast is a direct impression it is therefore an exact reproduction of the original specimen.

About 100 casts of dissections were demonstrated by us at the fifty-first annual session of the American Association of Anatomists ('35). Theses casts are permanent, extremely accurate, easily painted and are markedly resistant to chipping and scratching even though handled greatly.

Several commercial preparations for the negative impression or mold are readily available. They may be used several times and therefore their cost is moderate. Since the positive material is used but once and its price high it is the most expensive item in making permanent casts. In order to reduce this expense we have experimented with various combinations of ingredients to develop a substitute that would be inexpensive and as efficient as the best commercial preparations. A suitable mixture of materials meeting our requirements was finally obtained which is inexpensive, costing but a fraction of the price of the cheapest suitable commercial product. The preparation of this material is simple as only an enameled pan, Bunsen burner and brush are required. The ingredients are easily obtained and their total cost about one-tenth that of the commercial preparations.

The following formula was found most suitable in the production of a homogeneous, rather hard material for making positive casts.

		parts
Beeswax	(white)	10
Rosin	(white)	6
Zinc oxide	(powdered)	3
Talcum	(powdered)	2
Coloring material, Japan colors		

The measured amount of beeswax and rosin is placed in a large enameled baking pan. An enameled pan is used because of the ease in removing the prepared material after hardening. Care should be taken never to fill the pan more than three-quarters full. The beeswax and rosin are melted over a flame that is small enough to bring the mixture to just below the boiling point. If boiling occurs at this stage in the procedure it will be difficult to properly mix the zinc oxide and talcum with the beeswax and rosin. The material resulting from overheating is very granular, improperly mixed and contains many air bubbles. When the beeswax and rosin are completely liquefied the flame is reduced and about two-thirds of the measured amount of zinc oxide and talcum is sprinkled over the surface. Reduction in intensity of the flame is necessary at this stage in order to eliminate bubbles and prevent the mixture from bubbling over. A small brush rather than a stirring rod is used to stir the mixture. The brush removes the zinc oxide from the bottom of the pan better than a scraper or stirring rod. As soon as bubbling begins, the flame is removed entirely and the mixture stirred slowly until the danger of overflowing has passed. In most cases this is a matter of a few minutes. As soon as the bubbling has subsided somewhat, the heat is again applied and more vigorous stirring continued.

The mixture becomes somewhat darker in color and more uniform in consistency. The remainder of the zinc oxide is added and stirring continued until it is completely dissolved. The balance of the talcum is added and stirred until the mixture is homogeneous. The desired color is obtained by placing a small amount of the Japan coloring pigment on the brush and stirring it in. By adding small amounts of coloring matter any desired shade may be obtained. The flame is then removed entirely and the mixture allowed to cool slowly. The stirring is continued for a short time in order to remove any air bubbles. After cooling, the material can be removed from the pan by inverting and pressing upon its bottom. The positive material may then be broken into small pieces and used as needed by melting in a small container. It may be applied by pouring or by brushing into the negative mold. Since this positive material cools very rapidly (about 15 seconds), it is a very simple matter to build up successive layers as desired. The primary coating is then reinforced with pieces of cheesecloth. This is done by painting the cheesecloth into the mold with the positive material; this second coat passes through the meshes of the cheesecloth and fuses with the exposed surface of the first layer or primary coating of positive material. This primary coating is thicker than the subsequent reinforcing coats. The strength of the cast may be reinforced by merely increasing the number of layers of cheesecloth thus eliminating a third and expensive material when one uses commercial preparations. The finished cast is then gently removed from the mold and any defect remedied by either applying a heated scalpel and melting or by scraping away. The same material is used for patching and repairing purposes. These casts can very easily be painted with oil or Japan colors.

For purpose of comparison photographs of dissections and photographs of casts made from these dissections are included in this paper.

This positive material has been used in our laboratory for the past 2 years and has been most satisfactory. It cools very rapidly and is not adherent to the mold. It is completely devoid of air bubbles and has a smooth uniform texture.

The melting point, although not very high, is considerably higher than any temperature encountered in the hot summer months and there have been no signs of warping even though these casts have been in constant use. The material is not brittle and therefore does not chip or crack easily. Most important, however, from the standpoint of usuage in large quantities, is the extremely low cost and simplicity of preparing this positive material.

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PLATE 1

EXPLANATION OF FIGURES

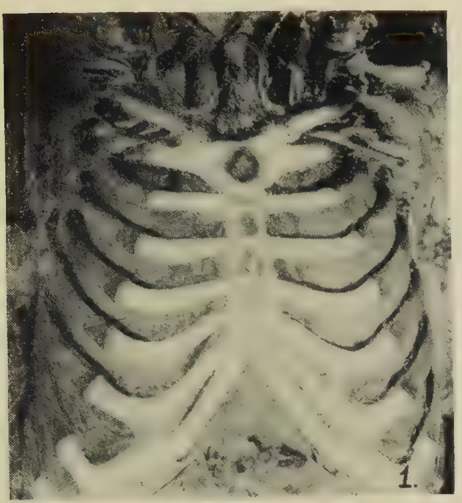
1 Dissection of 1-year-old infant. Skin and musculature removed from thorax to expose thoracic cage. Skin and superficial fascia removed to expose structures of neck. One-half natural size.

2 Cast of same dissection as in figure 1. Structures of neck, pleura and intercostal muscles on right side painted. Ribs, sternum and clavicle unpainted. One-half natural size.

3 Dissection; thoracic cage and musculature removed to show lungs, thymus and pericardium in situ. One-third natural size.

4 Cast of dissection shown in figure 3. Structures painted. One-third natural size.

5 Cast of abdominal dissection; anterior abdominal wall removed. Structures colored to resemble natural living condition. One-third natural size.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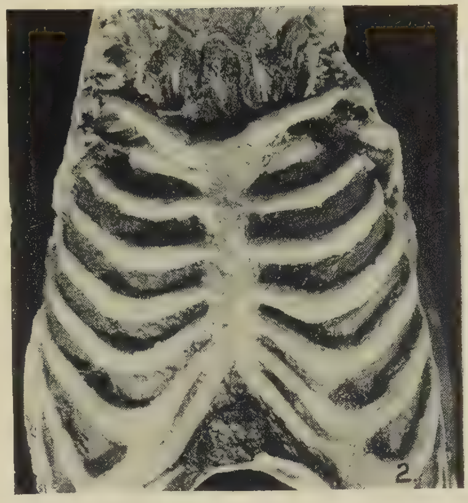
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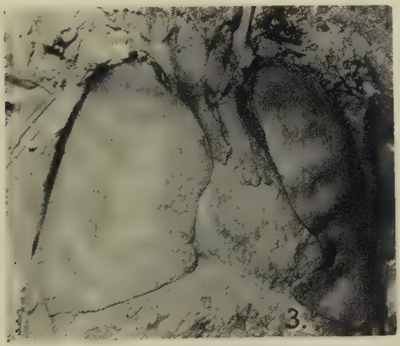
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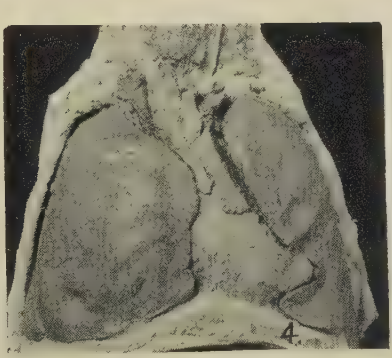
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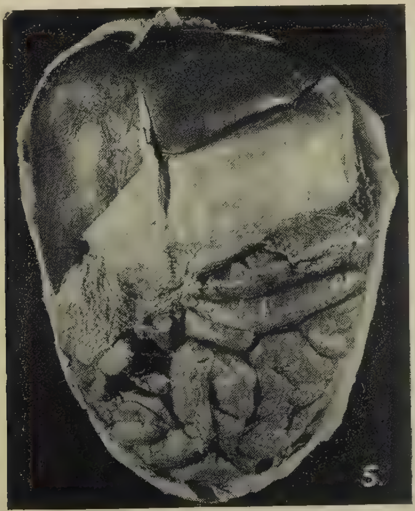
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AN ANENCEPHALIC HUMAN EMBRYO 16.5 MM. LONG

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FOUR FIGURES

The embryo herein described was secured from a spontaneous abortion by a woman 17 years old. It was brought to our laboratory soon after birth with the membranes intact. Inasmuch as we have been unable to find a recorded description of an early anencephalus of this type we feel justified in publishing an account of this embryo.

Its crown-rump length was 16.5 mm., and its external form, except for the anomalies of the head, was that of an embryo of about 7 weeks, being clearly in the transition from embryonic to fetal form. There was no conspicuous external evidence of decomposition. Microscopic study showed that there had been marked post mortem changes, but the condition of the tissues was still such that internal structures could be definitely identified.

The trunk and limbs were essentially normal, both externally and internally. The external ear was about as would be expected in an embryo of this stage of development. There was double harelip, and internal defects of palate and nasal septum.

The conspicuous deformity, as seen in figure 1, involved the upper portion of the head, which had a form simulating that of a brain consisting of cerebral hemispheres with prominent olfactory lobes and a cerebellum with two lobes. Microscopic examination showed this peculiar structure to be composed of highly vascular mesenchyme, quite loose within, but ending above in a thin, denser, non-cellular layer (see fig. 2, just below the word 'Marg'). At the lateral margin of this mesenchymal mass, the epidermal layer of the skin, consisting of a single layer of cuboidal cells, terminated in a ragged, partly detached edge, the location of which is shown by the dotted line in figure 1.

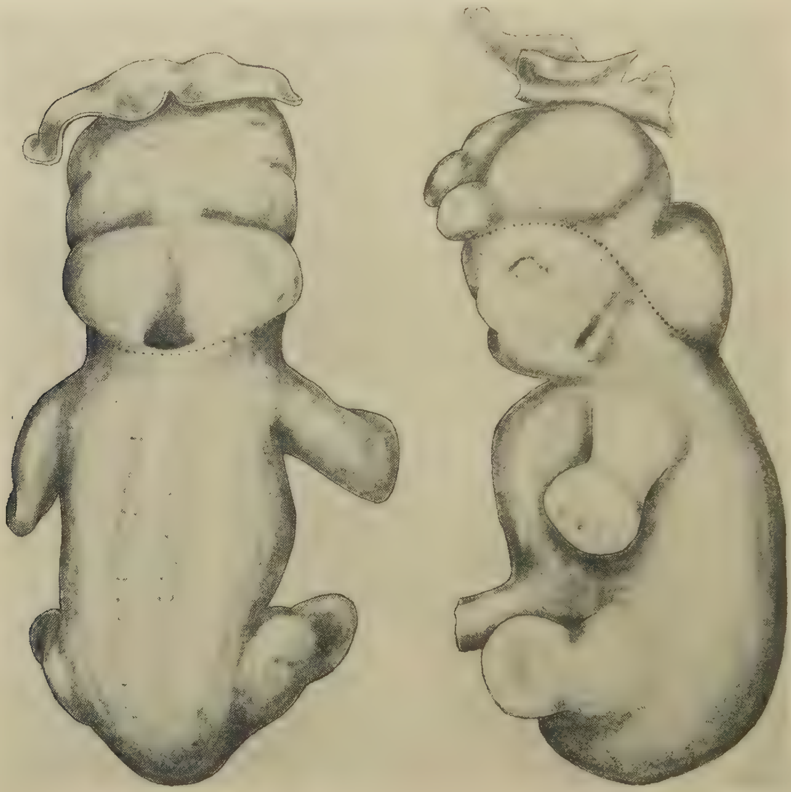


Fig. 1 Posterior and lateral views of anencephalic monster. Drawings made after preservation in formalin. Shows mesenchymal mass simulating the form of a brain. In it were seen many blood vessels before it was placed in formalin. The dotted line traces the torn edge of the epidermal layer of the skin. The irregular sheet of tissue attached to the top of the head is primitive nervous tissue. The opening into the spinal canal was filled with similar tissue.

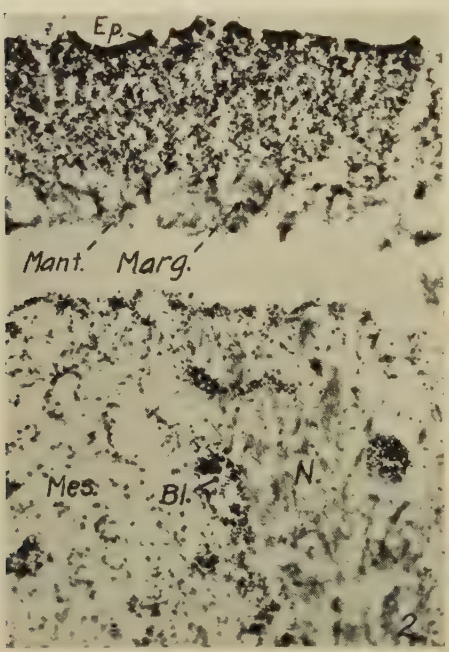
At the nape of the neck was a good sized opening leading downward into the spinal canal. From it there protruded a flocculent mass which became lost during the preparation of sections, but it was doubtless a broken down portion of the spinal cord. The persisting portion of the spinal cord was seen in sections to occupy the lower end of the spinal canal and to be in a fair state of preservation. At the margin of the opening into the spinal canal, the epidermal layer of the skin terminated, just as it did at the edge of the mesenchymal mass.

Probably the most significant feature of this embryo was a wrinkeled sheet of tissue loosely attached to the top of the head (fig. 1). Microscopic examination showed this to be composed entirely of primitive nervous tissue. Here and there were seen small fragments of this same tissue adhering to the surface of the mesenchymal mass.

This sheet of tissue had the typical structure of the primitive neural tube, consisting of a thin, highly cellular ependymal layer, followed by a thick mantle layer with less abundant cells, and a marginal layer with no cells (figs. 2 and 3). The ependymal layer faced outward, away from the head, while the marginal layer faced inward toward the mesenchymal mass, from which it had become separated at most places. This sheet of nervous tissue had a thickness of about 250 μ. Its histological structure was quite like that of the neural tube of the embryo as seen in the lower portion of the spinal cord, except that the spinal cord had become closed in the normal fashion.

We interpret the structures just described as indicating that the anencephalic condition of this embryo was produced by failure of the neural plate in the cephalic region to form a closed neural tube. It seems probable that the fragment of nervous tissue adhering to the top of the head is the remnant of the cephalic portion of the neural plate, which at one time covered the entire mesenchymal mass and was directly continuous at its margin with the epidermal (ectodermal) layer of the skin. It no doubt also extended downward into the spinal canal, where the neural groove had become closed to form the spinal cord.

We have no explanation for the great thickening of the mesenchyme underlying the cephalic portion of the neural plate, nor for its peculiarly suggestive form. It is evident, however that had this mesenchyme been of moderate, uniform thickness throughout its whole extent, the head of this monster would have resembled very closely that of the common form of full term anencephalic acranial monster, in which the cranial vault and brain are wanting and the covering of the flattened top of the head resembles the meninges without an epidermal covering. Certainly the condition observed in this embryo would furnish a reasonable beginning for such a condition should development continue to full term.



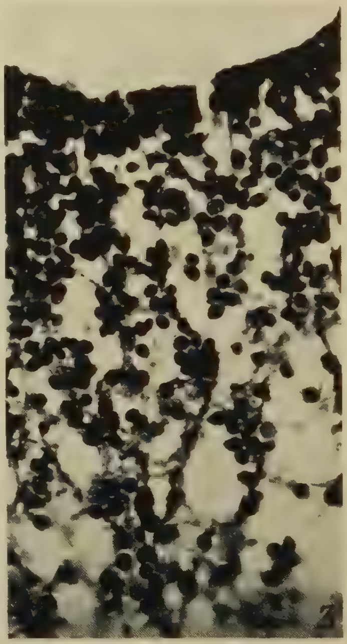


Fig. 2 Photomicrograph. × 90, through neural plate into mesenchymal mass. Shows ependymal, mantle and marginal layers of neural plate. In mesenchymal mass it shows blood vessels, and a nerve approaching the surface.

Fig. 3 Photomicrograph, × 400, showing structure of ependymal and mantle layers of neural plate.

Certain observations concerning the nerves and ganglia are of interest. The spinal cord was flanked by the spinal ganglia in the usual manner, and both dorsal and ventral roots of the spinal nerves were present. In the cervical region several ganglia were united into one elongated mass from which arose several nerves. These nerves, together with their corresponding ventral roots, united to form a rather imperfect cervical plexus. From this plexus arose the phrenic nerve (fig. 4).

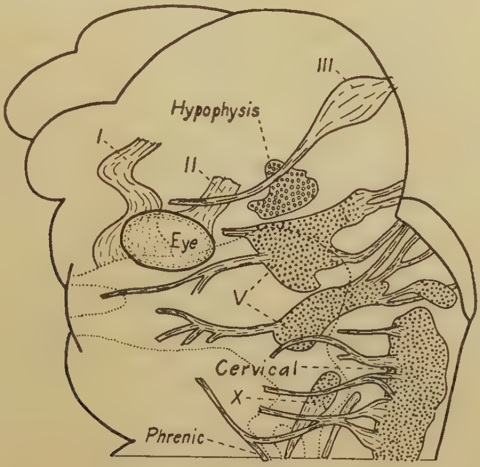


Fig. 4 Graphic reconstruction of head showing nerves and ganglia.

The cranial nerves and ganglia were traced with considerable certainty. In general it may be said that the cranial nerves had their apparent origins in the surface of the mesenchymal mass. Their fibers penetrated its surface by definite, foramen-like openings, and one of them, the oculomotor, actually connected with the nervous sheet where it was still in contact with the mesenchyme (fig. 4). Figure 2 shows a photomicrograph of one of these nerves as it approaches the surface. The following nerves were found.

The olfactory nerves had their origins in some fragments of nervous tissue lying in the fissure between the mesenchymal hemispheres. They coursed downward to the nasal mucosa.

The optic nerves could be traced backward from the eyeballs until they united in the optic chiasma, just as they pierced the superficial mesenchyme at the bottom of the median fissure. Within the eyeball the optic cup showed good development of both pigmented and retinal layers.

Just back of the optic chiasma, close to the nasal cavity, was a median mass of epithelial cells which we consider to be the anterior lobe of the hypophysis. We did not observe anything that seemed to represent the posterior lobe.

A pair of nerves without ganglia arose from the surface of the mesenchyme near the vertex, and coursed through the mesenchyme, past the hypophysis to terminate in the region of the eyeball. These are probably the oculomotor nerves. The enlargement on this nerve, shown in figure 4 is not ganglionic in nature and there is no corresponding enlargement on the other nerve of the pair.

In the region behind and below the eyes were two large pairs of ganglia which belonged to the trigeminal nerves. Each of them had several roots arising from the surface of the mesenchyme in locations shown in figure 4. The uppermost of these ganglia gave off two nerves which were evidently the ophthalmic and maxillary branches of the trigeminal nerve, while the other ganglion gave rise to the mandibular branch.

A smaller ganglionic mass connected with the second of these ganglia could not be identified.

In the lower pharyngeal region there were a pair of small ganglia from which arose nerves running into the thorax along with the sympathetic trunks, evidently the vagus nerves.

Neither ganglia nor nerve fibers representing the facial or glossopharyngeal nerves could be found, and there seemed to be complete absence of internal ear and auditory nerve and ganglion.

The presence of the ganglia of the trigeminal and vagus nerves in this embryo shows that there had been in the cranial region some development of the neural crest, or of the placodes which contribute to the ganglia, or of both.

It is of interest to note that the poorly developed neural plate had, in some measure behaved like a normal neural tube: From it had grown out the optic vesicles to produce optic nerves and retina. From it had also grown one pair of efferent nerves, the oculomotor. Deficiency of the neural plate is indicated by the absence of other efferent nerve trunks which if present ought readily to be recognized.

CONCLUSIONS

We believe that the anencephalic condition in this embryo arose by a failure of the neural plate in the cranial region to develop into a closed tube. Thus we explain the absence of brain and cranium.

The defect must have begun at least as early as the fourth week.

It seems reasonable to suppose that in at least the extreme types of full term anencephalic monsters, the mode of origin might well be just what is indicated in this early specimen, rather than the result of a 'catastrophe' or 'trauma' after the brain had become well formed. In this connection we believe that our specimen presents a crucial case.

SEXUAL RESPONSIVENESS AND TEMPORALLY RELATED PHYSIOLOGICAL EVENTS DURING PREGNANCY IN THE RHESUS MONKEY

JOSEPHINE BALL

The Henry Phipps Psychiatric Clinic and Carnegie Laboratory of Embryology, Johns Hopkins Medical School

ONE FIGURE

In connection with the investigations in the embryology and the physiology of reproduction being carried on at the Carnegie Laboratory of Embryology, the writer has been privileged to study the sex behavior of rhesus macaques whose physiological condition is being followed. It is the purpose of this paper to report the findings on three of these animals which became pregnant during the observations and to correlate quantitative changes in sexual responsiveness with certain anatomical and hormonal events which have been worked out by other members of the staff.

The method of measuring responsiveness was the same as that used in the study of cyclic changes in the normal, non-pregnant animal (Ball and Hartman, '35). It is a measure not of acceptance but of invitation. In this paper we showed that, while the female monkey may accept the male at any time in the menstrual cycle, there is, typically, a definite increase in sex interest around the middle of the cycle just before ovulation.

In figure 1 similar curves are given for a menstrual cycle of three females, the curves being continued into pregnancy. It will be seen that one of these animals was observed throughout pregnancy, one was surgically aborted on the sixty-ninth day of gestation and the third suffered spontaneous abortion on about the seventy-sixth day.

These curves indicate that sex interest drops after the rise before fertile ovulation, as in the non-pregnant cycle, to rise again 3 to 4 weeks later, almost as if conception had not taken place. This rise occurs soon after the beginning of the bleeding of pregnancy or 'placental sign' (Hartman, '29). After this, sex interest disappears, more or less gradually, and the animal remains quite indifferent to the presence of the male

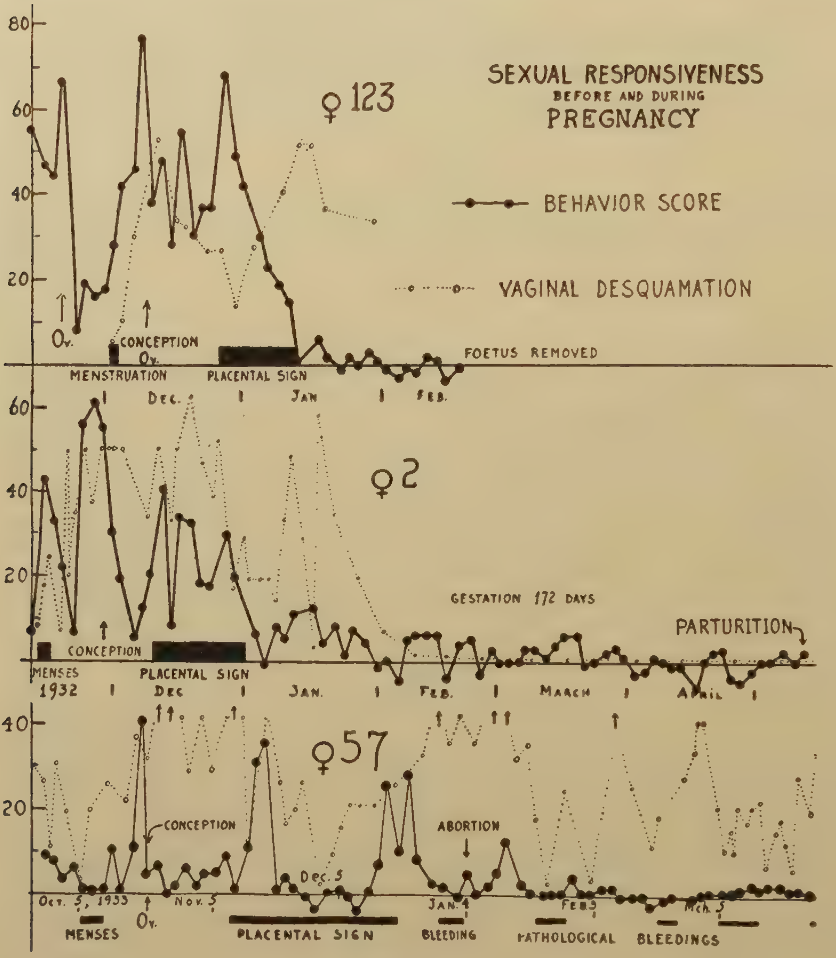


Figure 1

throughout the rest of a normal pregnancy. The variation from this shown by female no. 57, who aborted spontaneously, will be discussed below.

While this record of the macaque's sex behavior during pregnancy is a matter of some interest per se, it acquires somewhat greater significance when temporally correlated with certain other physiological processes, namely, the embedding of the egg, the excretion of prolan, involution of the corpus luteum, regression of the vaginal mucosa.

Unpublished data which are being gathered by Dr. G. L. Streeter and Dr. G. B. Wislocki (to be published in the Contributions to Embryology, Carnegie Institution of Washington publications) show that the attachment of the ovum to the uterine wall begins, in the monkey, between the ninth and tenth days after fertilization. The epithelium proliferates and is apparently ingested by the trophoblast. This process reaches its height at the fifteenth to sixteenth day, which is about the time the kidney begins to excrete the anterior pituitary-like substance (prolan) as has been found recently by Dr. G. W. D. Hamlett ('37).

By the twenty-fifth day of gestation the embryo has established the relation to the maternal structures which it maintains throughout the rest of pregnancy, obtaining its nourishment henceforth, not by direct ingestion of maternal tissue but by means of its now established circulatory system through the walls of the villi which have been developing from the sixteenth to the twenty-fifth days. It is on about the twenty-fifth day also that prolan practically disappears from the urine (Hamlett, '37), not to reappear in detectable quantities during the rest of gestation (Allen, Maddux and Kennedy, '31; Snyder and Wislocki, '31). This is also the date determined by Corner, Bartelmez and Hartman ('36) when the corpus luteum of pregnancy undergoes certain regressive changes.

Behavioral phenomena and the vaginal pictures are not so definitely timed and they seem to partake more of the nature of continuance of a cyclic tendency similar to that described in the pregnant chimpanzee by Tinkelpaugh ('33) and by Schultz and Snyder ('35) than to be an integral part of gestation, as are the events described in the last two paragraphs. There may or may not, therefore, be some significance in the fact that the peak of sexual responsivness comes between the sixteenth and twenty-fifth days. But it is certainly significant that this cyclic tendency is pretty definitely repressed after the twenty-fifth day when the primarily gestational processes have settled into their final form.

With reference to the changes in the vaginal mucosa, Hartman ('32) has described the continuation of cycles of desquamation in the first part of pregnancy. Usually there is a single rise after the one accompanying conception, corresponding roughly to the rise in sexual responsiveness. Occasionally there is a second wave and sometimes no rise at all. Desquamation in the three animals under discussion in this paper is shown by the dotted lines in figure 1, in per cents of sediment in the vaginal lavage (compare Hartman, '32). Female no. 2 had a second rise almost as high, if not so prolonged, as the first one. It will be noted that there is a suggestion of a rise also in sex interest. Possibly there would be cases of more definite irregularities in behavior as in desquamation had more cases been studied. Female no. 57, of course, constitutes a special case.

After the cycle or cycles of early pregnancy there is a progressive atrophy of the vaginal mucosa until, by the one-hundredth day of gestation, its condition resembles that of the castrate (Allen, '27; Davis and Hartman, '35), in which state copulation often causes bleeding (observations by the writer, to be published elsewhere).

The facts presented in this paper are insufficient for profitable speculation on the endocrinological significance of these temporally associated events. However, they make it evident that the first month of pregnancy is one of complex shifting of the endocrine controls, with a fairly abrupt settling into the organization that continues, apparently with relative stability, throughout the rest of the 5 months of gestation, although in some cases there may be one more wave of desquamation and possibly of sex interest after this first month.

With regard to the special case of female no. 57, there seems to be evidence in her curves of vaginal desquamation and of sexual responsiveness that this abortion was due to some sort of failure of balance in the endocrine complex that should control pregnancy, since there was a rise of sex interest before and an increase of desquamation before and after the probable date of abortion which was determined by palpation by Dr. C. G. Hartman. The stimulation of the vaginal mucosa of castrate monkeys has been described by Allen ('27) and by Davis and Hartman ('35). Increased sexual responsiveness has also been induced in castrated female monkeys by estrin injection (Ball, '36). These facts suggest that the abortion was due to an endocrine imbalance characterized by too much estrin. Because of the recognized antagonism between estrin and progestin, it is interesting to refer here also to the paper by Snyder ('34) who showed that parturition fails to occur in the rabbit until termination of the life of the corpus luteum, even when, by the induction of a fresh set of corpora lutea, gestation is prolonged to the point of uterine injury due to postmature fetuses. None of these facts, however, offer any clue to what started the endocrine imbalance that was the apparent cause of this abortion.

SUMMARY

Quantitative changes in sexual responsiveness which the writer has found in three pregnant rhesus monkeys are temporally correlated with other indicators of endocrine changes and with uterine events as they have been determined by various investigators in the Carnegie Laboratory of Embryology. The findings indicate that there is a complex shifting of endocrine controls throughout the first month of pregnancy while the embryo is establishing its relation to the maternal organism, with some tendency to continuance of cycles in behavior and vaginal desquamation.

In one case, spontaneous abortion was associated with a definite continuation of these cycles for 2 months after conception, indicating a failure of the endocrine mechanism which normally maintains pregnancy.

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THE FATE OF MOUSE OVA TUBE-LOCKED BY INJECTIONS OF OESTROGENIC SUBSTANCES[1]

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ONE PLATE (TWENTY FIGURES)

It has been demonstrated elsewhere that the injection of oestrogenic substances will cause the tubal retention of rabbit and mouse ova (Burdick and Pincus, '35; Pincus and Kirsch, '36; Whitney and Burdick, '36). In the mouse the daily subcutaneous injection of 7½ rat units of oestrone or oestrone benzoate invariably causes such retention when injection is begun on the day of copulation, whereas 5 r.u. per diem is a dosage that permits the occasional entrance of ova into the uterus (Whitney and Burdick, '36). In this paper we shall describe the nature of the development undergone by tube-locked ova.

The commercial oestrogenic compounds injected were Progynon, Progynon-B (in oil), Theelin, and Amniotin in the dosages indicated in table 1. The oviducts were teased or rolled out in a 0.5% saline solution. Mating time was determined by the presence of a vaginal plug.

As in the rabbit (Pincus and Kirsch, '36) neither the rate nor the mechanics of cleavage are affected by the comparison of the eggs of a large series of control and injected animals. Figures 1 and 2, for example, show ova recovered in the 6- to 8-cell stage from an injected female at 50 hours after copulation. These compare favorably with Lewis and Wright's

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[1] This work was made possible by grants from the National Research Council and the American Association for the Advancement of Science.

('35) figures 22 and 23 of plate 2. Similarly the 96-hour ovum of figure 3 resembles closely a similar stage shown in Lewis and Wright's figure 30, plate 3.

Normally the mouse ovum enters the uterus at 72 to 96 hours after copulation in late morula and early blastocyst stages (Enzmann, Saphir and Pincus, '32; Lewis and Wright, '35). Burdick and Pincus ('35) thought that tube-locked ova remained in the morula stage, but our data indicate that development into early blastocyst stages does occur. Thus the ova of figures 4, 5 and 15 are blastocysts of normal appearance in which the fluid accumulation into the blastocyst cavity has occurred exactly as described by Lewis and Wright ('35) for normally developing ova.

The zona pellucida of the mouse egg normally is lost from the expanding uterine blastocyst by the fifth day after copulation (Enzmann, Saphir and Pincus, '32). The disappearance of the zona in earlier stages has been recorded but only in fixed material (Huber, '15). We are in agreement with Lewis and Wright ('35) in believing that the fixing fluid probably dissolves the zona, and we have, in fact, observed such dissolution in eggs placed in Bouin's fluid. Tube-locked ova lose the zona pellucida at about the normal time and it is then that the first signs of developmental arrest ordinarily appear. These consist of a collapse of the developing blastocyst and the obliteration of the blastocyst cavity (figs. 4, 5, 8, 11, 14, 15 and 19). Degeneration may set in before the zona is lost (fig. 6) and such degeneration has been observed most frequently in ova recovered from animals receiving the higher hormone dosages (figs. 14, 15, 16 and 17). The beginning of ectoplacental cone formation does occur, however, in a number of the ova (figs. 7, 12 and 18), but progress beyond this stage does not occur. The ova finally collapse completely and the cells fragment off. This is illustrated in figures 5, 16 and 17 where the fragmenting masses are indicated by arrows. The process of degeneration is completed by the seventh day after copulation. We have rarely been able to obtain ova identifiable as such after the seventh day. The ova recovered by Burdick and Pincus ('35) at 10 to 14 days post coitum were probably from a second ovulation during this interval.

The process of collapse and fragmentation can be imitated by placing normal blastocysts in a hypertonic medium. Thus the ovum of figure 20 was so treated and its resemblance to the tube-locked blastocysts of figures 7, 8, 9 and 10 is obvious.

The implication from these observations is that the tubal fluids have a greater density than the blastocyst. It is notable that in order to preserve normal blastocyst form and appearance a 0.5% saline must be employed. In Tyrode's solution or a 0.9% saline collapse and shrinkage occur with some rapidity. The uterine fluids must normally attain a density suited for blastocyst growth and development. This is illustrated nicely in the ova of figures 12 and 13. The ovum of figure 12 was recovered from the right tube at 7 days after copulation, and the vesicle of figure 13 was flushed from the right uterus of the same animal which received a sub-maximal hormone injection (5 r.u. per diem). They were both photographed at the same magnification and the difference in degree of development is obvious. The ovum of figure 13 is in normal pre-implantation condition. Pincus and Kirsch ('36) (see also Pincus, '36), have clearly demonstrated that oestrone does not act directly upon the ova to inhibit development. The deduction from their and these experiments is that normal blastocyst development depends upon the presence of a special fluid environment in the uterus. The transition from a tubal to a uterine environment is not in itself sufficient for normal development as the collapsed uterine eggs of figure 9 indicate. Allen and Corner ('29) have shown that oestrin does not create this special environment in ovariectomized animals; corpus luteum is necessary. The nature of this corpus luteum hormone action is being investigated.

SUMMARY

Oestrogenic substances injected into mated female mice do not affect the early cleavage stages of the developing tubal ova. The normal round blastocyst stage, in which the trophoblast cells are in contact with the zona pellucida, is usually attained. Tube-locked ova ordinarily begin to degenerate at about the time when the zona pellucida is lost at the end of the round blastocyst stage. The tubal environment does not favor the continual growth of the blastocyst; collapse and fragmentation occur, and the ova disintegrate completely by 7 days after copulation.

We are indebted to the following for generous supplies of hormone: 1) Theelin, Dr. Oliver Kamm, Parke, Davis and Co. 2) Progynon and Progynon-B, Dr. Gregory Stragnell, The Schering Corporation. 3) Amniotin, Dr. C. E. Halstead, E. R. Squibb and Co.

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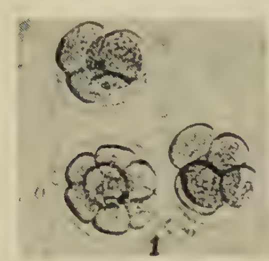
PLATE

TABLE 1 AND EXPLANATION OF PHOTOMICROGRAPHS

FIGURE	ANIMAL	HOURS AFTER VAGINAL PLUG	INJECTIONS IN RAT UNITS		LOCATION OF OVA	DESCRIPTION
			Daily amount	Total		
Progynon						
1	102	50	7.5 twice	37.5	Tubes	Ova in 6- to 8-cell stage
2	Same as above		7.5 twice	37.5		
3	114	96	7.5 twice	52	Tubes	Young blastocyst with zona
4	113	104	7.5 twice	52	Tubes	Two blastocysts: one with zona; other collapsed without zona
5	113	104	7.5 twice	52	Tubes	Blastocysts: one normal; two collapsed without zona; one fragment
6	51	104	7.5 twice	45	Tubes	Collapsed elongated blastocyst with irregular zona
7	60	120	5.0 once	25	Tube	Two collapsed blastocysts without zonas
8	27	120	5.0 once	25	Tube	Three late blastocysts, collapsed
9	Same as above		5.0 once	25	Uterus	Two late blastocysts: elongated and irregular. One with Träger?
10	65	6 days	7.5 once	45	Tubes	Two late blastocysts. With Träger?
11	52	6 days	7.5 first 2 days, twice thereafter	75	Tubes	Collapsed blastocyst
12	45	7 days	5.0 once	40	Tube	Elongated blastocyst without zona
13	Same as above		5.0 once	40	Uterus	Normal enlarged late blastocyst with Träger
Progynon-B						
14	145	75 hours	25.0 once	100	Tubes	Collapsed late blastocysts: one with zona. Debris?
15	Same as above		25.0 once	100	Tubes	Blastocysts: one normal; three collapsed with zonas; one without zona
16	124	120	25.0 once	100	Tubes	Late blastocysts without zonas; one collapsed and fragmenting
17	126	120	25.0 once	100	Tubes	Collapsed blastocyst, fragmenting
Theelin						
18	23	120	5.0 once	10	Tubes	Two elongated blastocysts without zonas
Amniotin						
19	8	120	5.0 once	25	Tubes	Two collapsed blastocysts; one fragmenting. Photomicrograph is of lower magnification than the others
20	83	102	Control—no injections		Uterus	Blastocyst collapsed because of density of medium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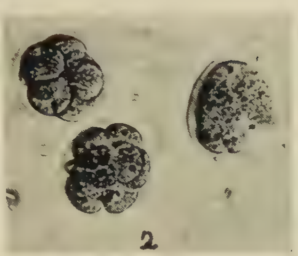
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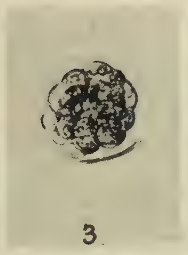
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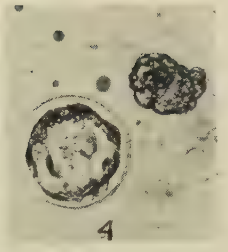
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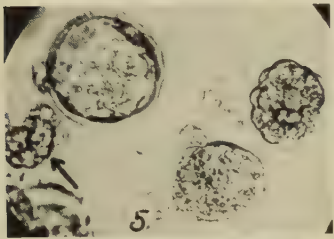
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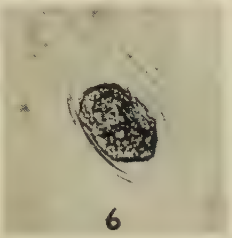
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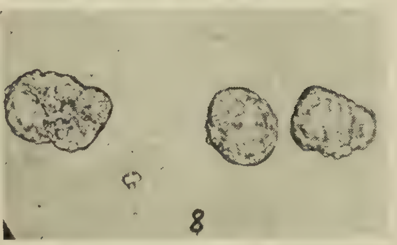
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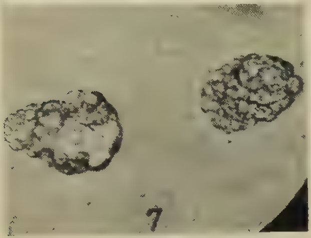
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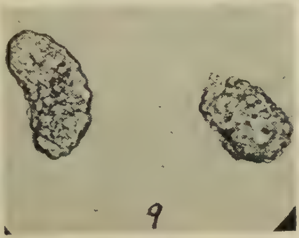
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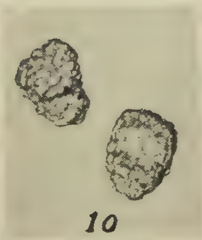
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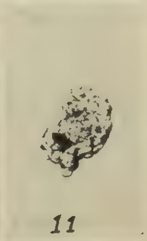
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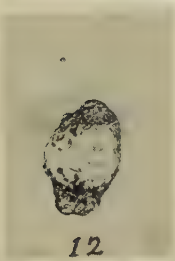
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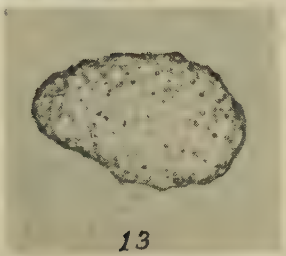
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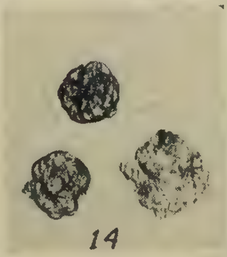
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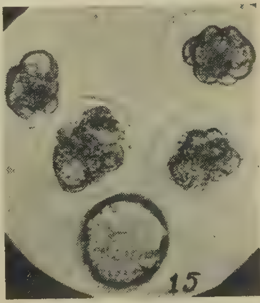
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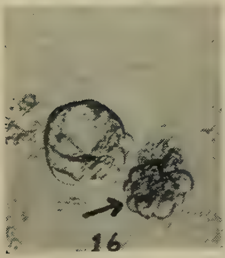
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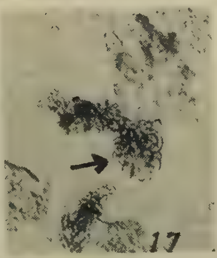
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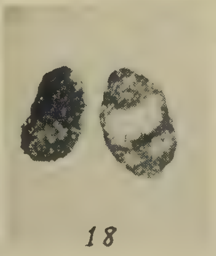
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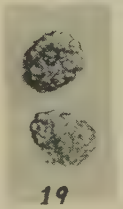
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STUDIES OF HEART MUSCLE IN TISSUE CULTURES

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ONE TEXT FIGURE AND TWO PLATES (FOUR FIGURES)

During the past 20 years much has been written about the growing of heart tissues in cultures. Many media have been used; chiefly salt solutions or some combination of plasma and embryonic extract. The various workers have not always agreed as to what types of cells other investigators have grown, though each one believes he has grown heart muscle in some stage of differentiation. We shall not attempt to review the literature, which has been so ably done by Levi ('34). Our contribution consists of the history of tissue cultures of embryonic chick heart muscle which have been grown for over 180 days in a liquid medium which is a combination of Tyrode solution, Locke-Lewis solution and embryonic extract. We have followed these cultures daily under the compound microscope, often with oil immersion, and have been able to observe details of cytoplasmic structure which we have later again studied in the same preparations after they were fixed and stained.

MEDIUM AND METHOD

The Locke-Lewis solution was made in the way described by Lewis and Lewis ('24) and was autoclaved. The Tyrode solution was the one used by Erdmann ('30). It was sterilized with a Seitz filter. We took 10 cc. of Tyrode solution and 2.5 cc. of Locke-Lewis solution and called it Tyrode-Locke-Lewis (TLL). This was used in making the embryonic extract. To one part of chick embryo crushed in a test tube (W. Lewis's method) we added two parts of TLL. We called this embryonic extract (EE).

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Tissue cultures were made from small pieces of the hearts of 8-day chick embryos in TLL. After 2 or 3 days this fluid was removed, the culture was washed in TLL and a culture medium containing one part of TLL and two parts of EE was added. After that the tissue cultures were washed in TLL and given fresh culture medium every 2 or 3 days until the cultures were 80 to 90 days old. Then the medium was changed every day.

The cultures were made on square cover slips in hanging drops over depression slides which had been ringed with salvoline (a mixture of petrolatum and paraffin). They were not transplanted as growth was comparatively slow after the first 4 or 5 days. One culture lived over 180 days during which time the medium was changed 106 times.

STAIN

To check up on our studies of living material, cultures from 7 to 180 days old were fixed in Susa solution and stained with Heidenhain's haematoxylin. A few cultures were stained with azan stain and a few were fixed in Champy's fluid and stained for mitochondria. The latter preparations were not satisfactory, due to the precipitation of the medium.

TEMPERATURE

The cultures were incubated at 37°C. and were studied in a warm box kept at about this temperature, because the rate of contraction of chick embryo heart muscle grown in tissue cultures is dependent on the temperature of the medium. When the temperature was below 33°C. the contractions became less frequent and soon stopped.

GROWTH OF THE CULTURES

In this culture medium 94.5% of the explants grew. Of these 65.4% contained growths of muscle cells. When the cultures are a few hours old fibroblasts begin to wander out. In 48 hours there is a large growth of fibroblasts. From 24 hours on until the death of the culture, muscle cells may be found migrating from the explant. They occur in large masses (fig. 1), in small groups, or as isolated cells along the side of the explant. They first appear as single cells, as in Locke-Lewis medium, but as they grow out they seem to be pulling a whole network of muscle cells with them. As the fibroblasts move away from the explant this network of cardiac muscle cells becomes increasingly clear, especially when one uses a binocular compound microscope. Then the connection between the muscle cells in the explant and those outside is very evident. In other words, the growth of cardiac muscle cells in this medium is largely, though not wholly, a migration of the cells en masse from the explant and their flattening out on the cover slip. This migration of muscle cells is helped by the more rapid migration of the fibroblasts which make room for the slower moving muscle cells.

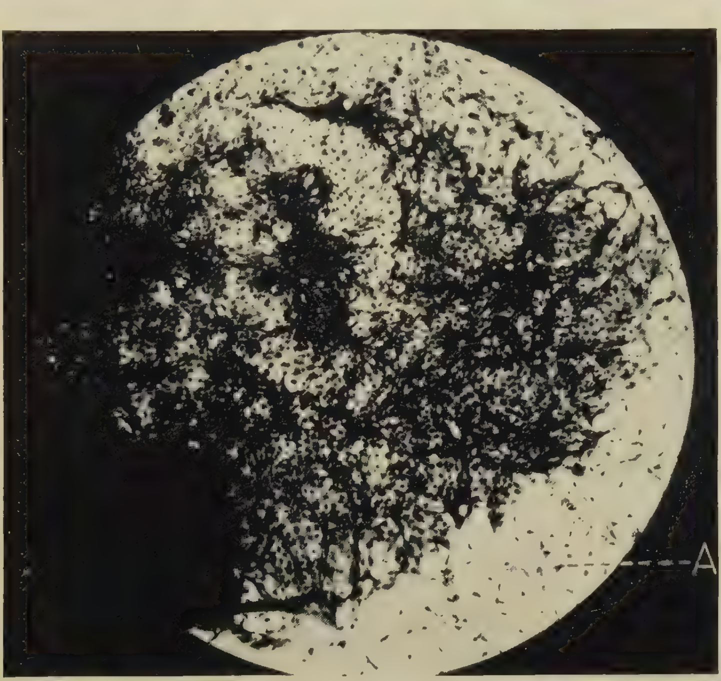

Fig. 1 Photomicrograph of a 48-day-old culture of cardiac muscle from an 8-day chick embryo. A, fibroblasts. × 50.

In older cultures (40 to 50 days old) after many of the fibroblasts have wandered out we sometimes saw elastic fibers twisted about among the cells remaining in the explant. Occasionally these fibers were present in the network of muscle cells which had grown out from the explant.

In the living cultures it is usually easy to distinguish a network of muscle cells from a mass of fibroblasts. The muscle cells, both in the explant and outside, form a definite network with round or oval spaces between the cells (figs. 2 to 4). The migrating fibroblasts on the other hand either form a plate of closely packed cells usually elongated in the direction of their migration, or they migrate quite separately from each other (fig. 1, A).

Furthermore, the muscle cells and fibroblasts have many points of difference. In the muscle cell the nucleus is usually round with one round nucleolus as described by Lewis ('26) and others, while in the fibroblast the nucleus is usually oval and mostly contains two nucleoli which practically always have irregular outlines, especially in the living cells.

In these experiments the cells within the explant and those outside seem to be exactly alike except that those at the edge of the new growth are flatter than the others (fig. 3). In our fixed preparations we find cross striations in cells just coming out from the explant (fig. 4), as well as in those farther removed from the explant (figs. 2, 3). We do not find any evidence that, in this medium, there is any dedifferentiation of muscle cells, as has been advocated by many investigators for cells grown in other media. Here the muscle cells remain muscle cells and the fibroblasts remain fibroblasts.

Nor do the muscle cells in the explant necessarily degenerate, as stated by others. Contraction continues in the explant long after the cells which have migrated from it are dead. Sometimes from these explants in which pulsations have been going on for weeks, without other signs of life, there may occur a migration of fibroblasts and muscle cells.

HISTORY OF THE OLDEST CULTURE

Our oldest culture of cardiac muscle cells lived for 180 days. It began in the usual way already described, fibroblasts appearing first, then muscle cells. When the culture was 30 days old there were only a few living fibroblasts in the outgrowth, as many had died. There was still a beautiful network of muscle cells which were contracting with the explant. When 41 days old the muscle cells which had migrated from the explant had all died but there was still a good growth of fibroblasts. After that the migration of fibroblasts became less and less, while the muscle cells continued to grow out in a network from the explant. As the outermost muscle cells died, new ones grew out in their places. These all contracted.

In the meantime, debris from the dying cell was accumulating. We cut some of this away with an iridectomy knife leaving a clear open space into which the new cells migrated and where it was easy to follow them under the microscope. The large muscle cells grew out slowly. Often fibroblasts were adhering to them. When the culture was 180 days old the cells were becoming granular. We fixed it on the 182nd day but all the cells were dead. We had last seen them contract on the 158th day. In another culture which was 98 days old when fixed the muscle cells were striated.

UNINUCLEATE CELLS

So far our studies of cardiac muscle in living and fixed preparations lead us to believe that most of the cells are distinct and uninuclear. In some of our preparations (figs. 2, 3, 5) of 48- and 55-day cultures where growth was extensive we found many binucleate cells. These we consider to be stages in cell division. There are a few places in our prepared slides where what appears to be a multinucleate mass of cytopblasm, in which cell boundaries are not clearly distinguishable, is seen migrating from the explant. Such structures are not seen farther out in the new growth and this apparent absence of cell boundaries is probably due to the crowded conditions near the actively growing edge of the explant. As a rule the nuclei are fairly evenly distributed in the network of muscle cells.

MYOFIBRILLAE

In the living contracting cardiac muscle cells we have seen distinct myofibrillae with cross striations, in bundles and singly. Sixty-five per cent of our stained preparations show this cross striation (figs. 2 to 5). In many of our preparations the Q, J and Z bands and Hensen's disk are visible (fig. 5). The striations are seen in the cells as they emerge from the explant (fig. 4) and in the cells forming the network outside (figs. 2, 3, 5). As the muscle cells flatten out these striated myofibrillae become more distinct (fig. 3) but they tend to disappear at the free edge of the outermost cells.

In many of the preparations the myofibrillae seem to pass from cell to cell (figs. 2, 5) but like Lewis ('26) we interpret this as the overlapping of their long thin processes which may cover several cells. This is quite evident when myofibrillae from different cells are seen crossing each other over the same cell.

In both the living and fixed preparations we have observed wavy and straight myofibrillae. The cross striations in wavy myofibrillae, though present, are not as distinct as in the straight myofibrillae. We believe that the myofibrillae are straight when the cells are either advancing or contracting, in other words, when they are under tension. The wavy fibrillae are seen in cells in the middle of a network (fig. 2, a) i.e., in cells not under great tension. Our impression is that the myofibrillae contract with the cytoplasm of the contracting cell (de Rényi and Hogue, '34).

CONTRACTION

When the explants were put in a hanging drop they usually beat rapidly for 1 or 2 days. Gradually the rate of contraction decreased. Sometimes contraction stopped entirely, or was confined to a small part of the explant. In a few cases the entire explant continued to beat slowly. This was the period of great fibroblast outgrowth. When the cultures were about 14 days old the rate of contraction began to increase. Both the explant and the muscle cells which had grown out from it began beating either at the same rate or at different rates.

There was great individual variation. A 14-day culture beat thirty-one to thirty-two times per minute at 38°C. A 17-day culture beat 180 to 244 times per minute at 36°C. and 224 times at 38°. A 33-day culture was beating 152 times per minute at 32° when it suddenly stopped beating. A 48-day culture beat 136 times at 37° and 184 times per minute at 39°C.

In one culture the muscle cells which had been beating regularly for several hours changed their rate of contraction. In another culture the cells which had been contracting regularly for several hours suddenly stopped contracting. This culture was returned to the incubator. Half an hour later the muscle cells were contracting again.

These changes in the rate of contraction may have been due to lack of food and oxygen or to the accumulation of waste products or to the effect of the light used for illumination.

As the cultures grew older the rate of contraction became much slower. A 139-day culture beat two to three times per minute at 29°C. and five to six times per minute at 35° when it was 143 days old. One old culture which lived to be 98 days old (when it became infected and was killed) would begin to beat as soon as it was given fresh medium.

Like Lewis ('26) we had cultures where two cells, close together in a network, were beating at different rates. We also had large growths of muscle cells coming from different parts of the same explant which beat at different rhythms in different parts of the network.

Many of our preparations contained two explants from different parts of the heart. These beat at different rates though they were often connected with each other by growths of fibroblasts.

Sometimes cultures which were beating when they were first made would go for weeks without contracting. Then they would begin to contract regularly, and as already mentioned explants whose cellular outgrowths had all died, would often continue to beat for days although there was no longer migration of cells.

FIBROBLASTS

The fibroblasts did not live in this medium as long as the muscle cells. Frequently they all died leaving a distinct network of muscle cells. In our older cultures (90 to 180 days) a few fibroblasts wandered out but they did not contain many granules or fat globules. At first they migrate and multiply more rapidly than the muscle cells do.

DISCUSSION

We have been fortunate in finding a simple medium of salt solutions and embryonic extract in which we could follow the activities of the muscle cells for 180 days. Other investigators who have kept the heart muscle cells alive over a long period of time have used either plasma or serum. Some of them have studied the contractility of the explant, others the contractility of the muscle cells which have migrated from the explant. Burrows ('13) kept chick embryo heart muscle cells beating in 'wick cultures' for 28 days in freshly prepared blood serum. Olivo ('30) studied the contractility of cultures from 3-day chick embryos for 6 months. Hoppe ('28) kept pieces of heart from guinea pigs 2 cm. long beating for 2 months. The cultures died after 14 weeks. Carrel's ('21) culture from the heart of a 7-day chick embryo which he started in 1912 is composed only of fibroblasts. Some writers claim that these were originally muscle cells. Both Levi and Olivo ('28) think that the muscle cells dedifferentiate when cultured in vitro. From our experiments we are convinced that in this medium the muscle cells of 8-day chick embryos remain muscle cells and the fibroblasts remain fibroblasts.

In regard to contractility Nordmann and Rüther ('31) have shown that many factors enter into the heart beat: a) the region of the heart from which the explant is taken, b) the age of the culture and c) the medium used. We used the same medium for all our cultures but in addition to factors *a* and *b* we found that the temperature of the tissue culture was very important, for as the cultures grew cool the beat became slower and soon stopped.

Some investigators have found it difficult to see myofibrillae in living muscle cells. In this medium where the cells spread out very flat and where there is no thick plasma clot the individual muscle cells can be clearly seen with their myofibrillae containing light and dark bands. These same places have been seen later in fixed preparations where the Q, J and Z bands and Hensen's disks were very distinct.

SUMMARY

1. The culture medium contains Locke and Tyrodes salt solutions, chicken boullion and embryonic extract. In it 94.5% of the explants of 8-day chick embryo heart grew; 65.4% of these showed growth of muscle cells; 65% of the muscle cell cultures contained cross striated myofibrillae when fixed and stained.

2. Cardiac muscle cells have lived in it 180 days. They have maintained many of their structural peculiarities and have appeared to function regularly. No evidence was found for the dedifferentiation of muscle cells grown in this medium.

3. Myofibrillae with cross striations have been seen in living cultures and in the same preparations after they were fixed and stained.

4. Most of the cardiac muscle cells are uninuclear, a few are binuclear and a very few multinuclear.

5. The rate of contraction of the cardiac muscle cells and the factors affecting it have been studied.

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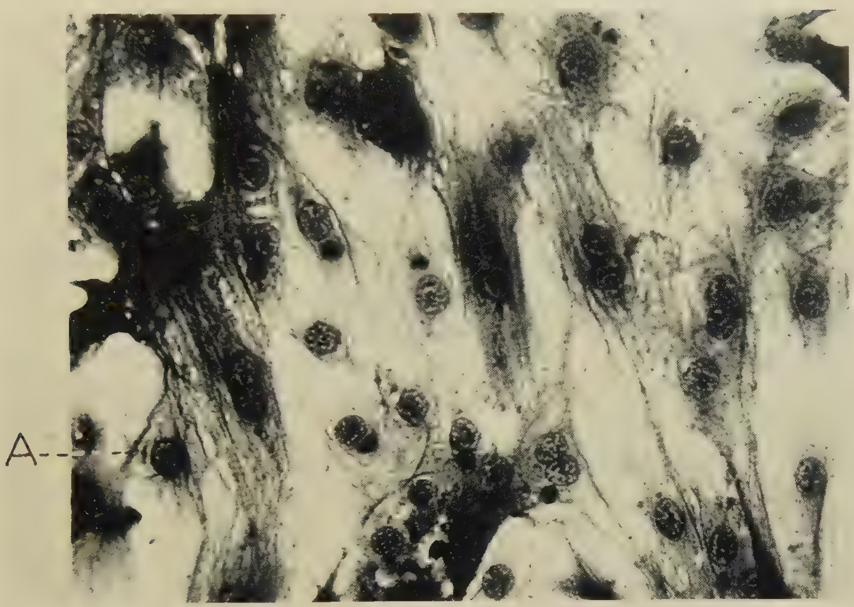
PLATES

PLATE 1

EXPLANATION OF FIGURES

2 Photomicrograph of cardiac muscle from 48-day culture, showing binucleate cells and wavy (A) and straight myofibrillae. × 530.

3 Photomicrograph of cardiac muscle cells at the edge of 48-day cultures. × 530.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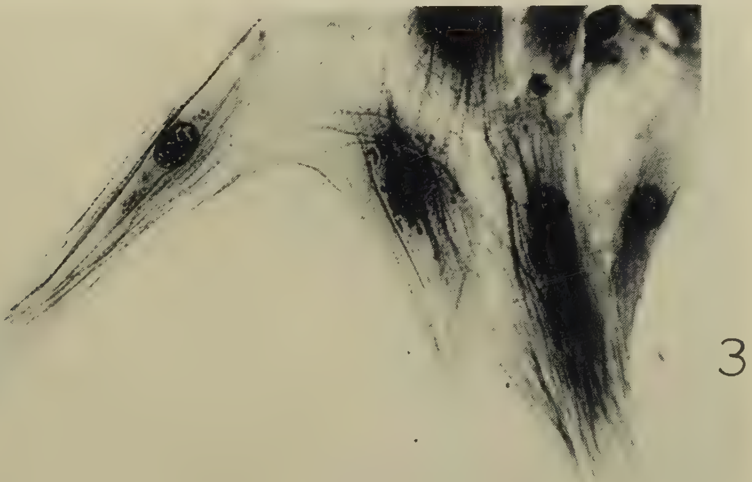
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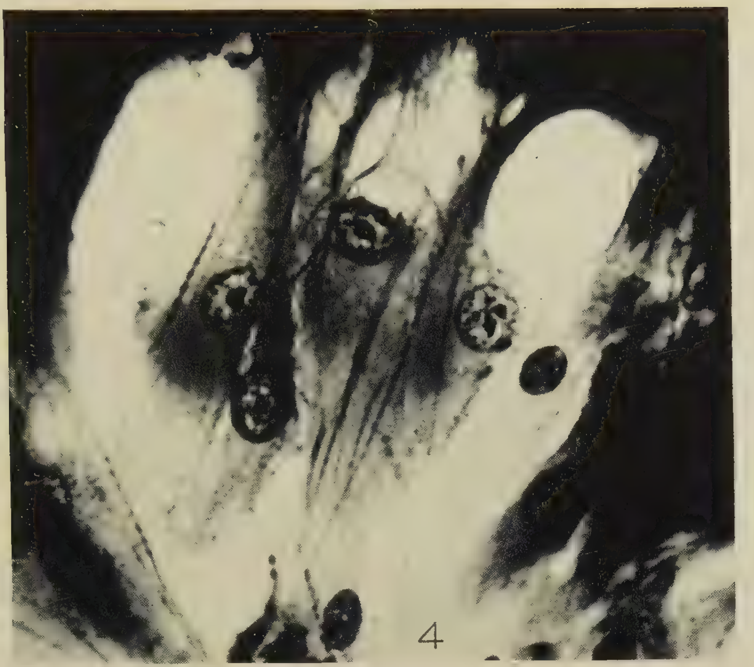
PLATE 2

EXPLANATION OF FIGURES

4 Photomicrograph of muscle cells, with striated myofibrillae, migrating from the explant. From 48-day culture. × 1330.

5 Photomicrograph of myofibrillae showing bands Q, J and Z and Hensen's disk from 55-day culture. × 2660.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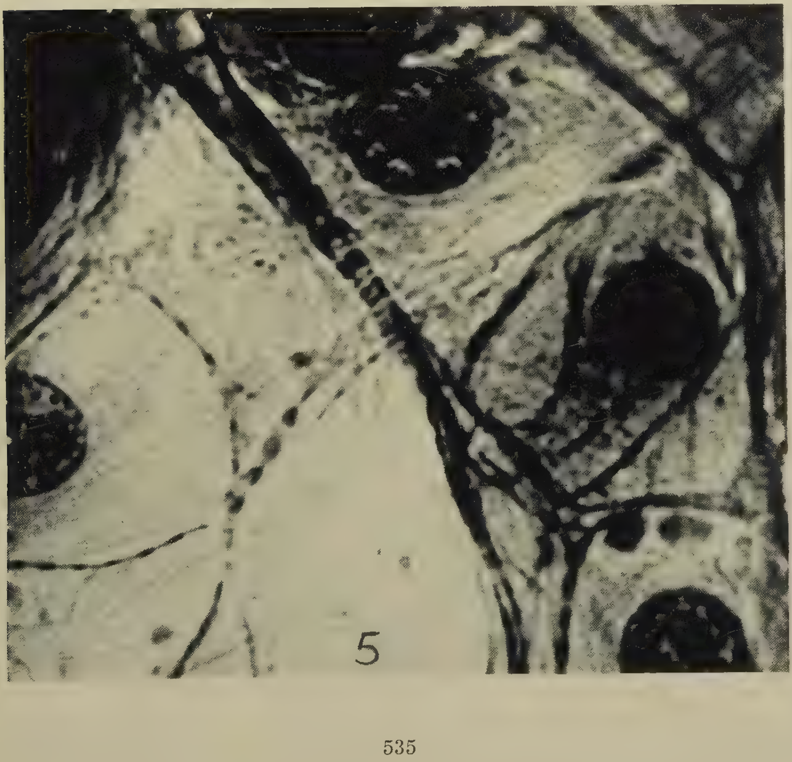
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NEW BOOKS AND PUBLICATIONS

THE LUNG, by William Snow Miller. 1937. Size 6½ × 10 inches. xiv + 212 pages. 152 illustrations, 20 of which are in colors. Index. Bibliography. Cloth. Price $7.50. Charles C. Thomas, Springfield and Baltimore.

An outstanding feature of this volume on the anatomy and histology of the lung is the excellence of its illustrations. Photographs, line drawings and colored plates have been successfully used to illustrate the text and to make lung structure understandable. In addition to descriptions of all parts and tissues making up the lung, there is an historical review of the literature, from Vesalius to the present time, dealing with air spaces in the lung and their arrangement.

PHYSIOLOGIE GÉNÉRALE DES ARTICULATIONS A L'ÉTAT NORMAL ET PATHOLOGIQUE, by A. Policard. 1936. Size 6¼ × 9¼ inches. 214 pages. 25 illustrations. Index. Bibliography following each chapter. Price 36 francs. Masson et Cie, Paris.

Joints in general rather than any particular joint are described in this book. It shows the construction and working of the normal articulation, together with an account of anatomical and histological changes taking place in the different tissues as disease develops. The component parts of a joint are described in the following order: Articular cartilage; fibro-cartilaginous coverings to the articular surfaces (marginal bourrelets and menisci); synovial membrane lining the articular cavity; articular cavity and synovial fluid; capsule and ligaments; innervation of the joint and periarticular connective tissue.

DAS HORMON DES CORPUS LUTEUM (BIOLOGIE, CHEMIE UND KLINIK), by Erich Fels. 1937. Size 7 × 10 inches. viii + 169 pages. 40 illustrations. Subject and author indices. Price RM 14.40. Franz Deuticke, Wien and Leipzig.

This book supplies a summary of research and investigations on the hormone of the corpus luteum. It includes an outline of the history of research, tests, standardization, chemistry and synthesis, relation to other hormones, action on genital system of both sexes, effects on various physiological activities and clinical use of the hormone.

ITALICA MEDICINA, Vol. I, No. 1. Size 7 × 10 inches. 64 pages. Yearly subscription, $5.00; single copies $0.50. Italian Medical Center, New York.

A new publication sent out its first issue in January 1937. It is the organ of physicians of Italian extraction practising in the United States, and describes itself as a "Monthly journal designed to be a bridge between the Italian and the American Medical Professions." The first issue contains editorials, historical matter, original articles and book reviews.

DIE COMMISSURA ANTERIOR DER WEISSEN RATTE, by Georg Kreiner. 1936. Reprinted from Zeitschrift für Anatomie und Entwicklungsgeschichte, vol. 106. 15 pages. 4 illustrations. Julius Springer, Berlin.

PLEXUS COROIDEOS DE LOS VENTRICULOS LATERALES, by Ernesto Danied Andia. 1935. 94 pages. 61 illustrations. Hospicio de las Mercedes, Buenos Aires.

RAFE VERTEBRAL, Acerca de un elemento óseo del conducto neural, by Ernesto Andia. 12 pages. 28 illustrations. Hospicio de las Mercedes. Buenos Aires.

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FIFTY-THIRD ANNUAL SESSION

University of Toronto, Toronto, Canada

March 25 to 27, 1937

ABSTRACTS

PAPERS TO BE PRESENTED

(The numbers in parentheses indicate the order in which the papers appear on the program of the meeting; the numbers in italics give the serial number of the abstract as printed herewith in alphabetical order of the authors' names.)

(147) *1. The activities of living blood capillaries in relation to the absorption of urea.* Richard G. Abell, Department of Anatomy, University of Pennsylvania.

Quantitative chemical studies show that following the installation of urea solutions within moat chambers, the rate of diffusion of urea into and removal by the blood capillaries is directly proportional to the diffusion gradient and to the absorbing capillary area. Microscopic studies indicate that subsequent to introduction of 0.2% urea solutions into moat chambers, an increase in the number of blood capillary sprouts formed by the capillaries most directly exposed to the solutions occurs. Such an increase has been found to take place when the concentration of urea in the extravascular reservoir, or moat, has fallen to 0.12% to 0.14%. Higher concentrations inhibit sprout formation.

When the concentration of urea in the moat decreases to approximately 0.06%, sticking of leukocytes in the capillaries closest to the urea solution in the moat is normal (phase 1). An increase in the concentration to 0.07% to 0.08% results in an increase of sticking to phase 2, possibly even phase 3, while an increase to 0.2% is followed by an increase in leukocyte sticking to phase 4, with emigration. A 1.0% urea solution causes small hemorrhages, but destruction of the capillaries (phase 6) does not occur until the concentration of urea in the moat approximates 5.0%.

Associated with the softening of capillary endothelium caused by the higher concentrations of urea is an increase in diameter of the supplying arterioles.

(41) *2. The reaction of certain endocrine glands of immature female mice to treatment with anuran anterior pituitary substance.* A. Elizabeth Adams and Gertrude R. Tukey, Department of Zoölogy, Mount Holyoke College.

Studies of the endocrine glands of mammals after administration of amphibian anterior pituitary substance are rare and the results mainly negative. In the present experiments, suspensions in salt solution of fresh or acetone-dried anterior pituitary lobes of Rana pipiens during the interbreeding season (October through February) were injected into 18- to 19- and 21- to 22-day-old female mice. Female frogs were used as donors in all but three of the thirty-five mice treated since their pituitaries are reported to be twice as potent as those of males in inducing extra-seasonal ovulation in frogs (Rugh, '34, '35). The numbers of pituitaries given per animal ranged from sixteen to ninety-six. Litter mate controls received fresh or acetone-dried frog brain tissue in salt solution. The injections were given during 4 days and the mice killed 96 hours after the first injection. Vaginal smears were made before killing. Ovaries, uteri and vaginae, thyroids and adrenals were dissected out, weighed and fixed for histological study.

The reaction of the reproductive system to the anuran pituitary substance was negative; that of the thyroids and adrenals positive. Both the absolute weights and the ratios of organ weight to body weight of the latter glands were increased and histological evidences of stimulation were apparent. No reaction of ovaries, thyroids, or adrenals was observed after injections of frog brain tissue.

(16) *3. The vascular relations of the glomus caroticum in the dog.* W. H. F. Addison and J. H. Comroe, Jr., University of Pennsylvania.

The carotid body in the dog is found closely attached to a small branch of the occipital artery, or to a small vessel arising directly from the dorsal surface of the external carotid artery about 5 to 8 mm. above the division of the common carotid into external and internal carotids. In either case, the body is associated with the mesial surface of this vessel usually 5 to 7 mm. from the external carotid, where the carotid sinus nerve fibers cross it.

At this point the carotid body vessel, which until here has the structure of an artery, loses most of its middle coat and assumes a vein-like appearance. The carotid body is attached to this vessel, immediately after the arterio-venous transition. The channels are in communication with the lumen of the vein by multiple connections. Elastic tissue is absent from the walls of the small blood channels of the glomus, but appears in the walls of the larger channels as they approach the wall of the vein. It is suggested that the myoid elements of the glomus are the derivatives of the cells which would have formed the muscle fibers of the tunica media of that part of the vessel from which the glomus developes.

(143) *4. The neurone as studied by micro-incineration.* Leo Alexander, Department of Neurology, Harvard Medical School. (Introduced by D. M. Rioch.)

The micro-incineration method, first conceived by Raspail, developed and perfected by Policard and Scott, is capable of contributing some data concerning the structure of the neurone. The two main points are: 1) intraneuronal and 2) interneuronal differences in the content of heat resistant ash.

Intraneuronal differences. Ganglion cells contain considerable mineral deposits in their nucleolus, their cytoplasm and in their dendrites. The nucleus is essentially free from mineral residue except for some faintly visible strands especially along the nuclear membrane. The mineral deposits in the cytoplasm are globular and granular in character and correspond in their arrangement to the Nissl bodies. They contain calcium, very little iron and some water soluble minerals. The intracellular neurofibrillae, the axone hillock and the axis cylinder, however, are free from heat resistant mineral ash. Only in certain ganglion cell diseases, such as Alzheimer's disease, the neurofibrillar structures are found to contain heat resistant mineral ash. (We found only one other type of mammalian tissue completely free from such mineral, namely, the elastic membranes of arteries).

Interneuronal differences. Differences in amount, quality and quantity of mineral deposits are not only found in ganglion cells of dissimilar morphology, such as between spinal ganglion cells, pyramidal ganglion cells and anterior horn cells, but also between ganglion cells which are, when studied with the ordinary staining methods, of very similar shape and structure, such as the granular cells of the cerebrum and the cerebellum respectively. The granular cells of the cerebellum are extremely rich in mineral deposits, calcium as well as iron, while cortical granular cells from the fourth layer of the iso-cortex are very poor in mineral residue. In the olfactory bulb two types of granular cells may be distinguished; one poor, one moderately rich in mineral residue. The large cells of the allo-cortical areas are, on the whole, richer in mineral deposits than cells of similar type from iso-cortical areas. There is a great deal of variation in the mineral distribution corresponding to architectural fields.

(71) *5. Growth of ovaries and genital tract in response to hormones as studied by the colchicine technique.* Edgar Allen, George M. Smith and William U. Gardner, Department of Anatomy, Yale University School of Medicine.

Use of colchicine, which arrests dividing cells in metaphase, accentuates evidence of growth (cell division) in genital tissues stimulated by hormones. The number of mitoses resulting from injections of theelin in ovariectomized adult mice was most pronounced in genital epithelia (Anat. Rec., Dec., 1936).

A second experiment in normal prepuberal mice with larger dosages of theelin gave an extremely high incidence of mitosis in uterine muscles, endometrial stroma and vascular endothelium.

The ovaries of normal mice and of immature mice injected with F.S.H. contained rapidly growing follicles with from 30 to 40% of the follicle cells in mitosis. The theca of growing follicles also has a high mitotic index, indicating that rapid growth of this zone is a factor in approach of the follicle to the surface in preparation for ovulation. Endothelial mitoses are frequent as early corpora lutea become vascularized.

Ovogenesis during sexual maturity becomes clear with the colchicine effect. Mitoses in the germinal epithelium, the first stage of ovogenesis, may number more than 1000 per mouse ovary at oestrus. Formation of new cells from the epithelium is cyclic, indicating some dependence of new generations of ova on maturition of preceding ones.

The use of colchicine accentuates mitotic division in tubal epithelium and in sprouts of mammary ducts. It is a splendid technique for the study of hyperplasias.

(69) 6. *Neurological interpretation of fetal behavior. The development of the sensory system in albino rat.* A. W. Angulo y Gonzalez, The Wistar Institute of Anatomy and Biology.

A study of the histogenesis of the sensory system was undertaken in an effort to ascertain the sequential order of the development of this system. While this work is not, as yet, complete, a number of facts have been learned which permits the presentation of the following statements:

a. The sensory system follows the motor system in development.

b. The earliest sensory endings to develop appear in the region of the snout. These earlier sensory endings are of the type called free-endings and precede in appearance the formation of hair follicles and the specialized nerve-endings which differentiate around them.

c. Nerve endings develop in the region of the fore limb much later than those of the snout.

d. The fibers that form the dorsal roots of the spinal ganglia enter the spinal cord and bend cephalad to establish connection with the nuclei gracilis and cuneatus, and in the younger fetuses these fibers do not send off collaterals in their course.

e. Later collaterals appear in this system and enter into relationship with the spinal motor centers.

These anatomical facts will be discussed in order to show that they substantiate my physiological findings relative to the development of behavior in the albino rat. (See demonstrations nos. D3 and D4.)

(50) 7. *The air-cells of the petrous portion of the temporal bone.* Barry J. Anson and John P. Gaardsmoe, Department of Anatomy, Northwestern University Medical School.

Numerous reports have been made in the past on the air cells of the petrous part of the temporal bone. These reports have dealt with the size, location, and communications of the cells. They have been based upon studies of gross sections, roentgenograms, dissections and corrosion preparations, and not upon reconstructions prepared by the method of Born—which alone permits of establishing their relationships and positively distinguishing them from marrow spaces.

By the wax-plate method we have reconstructed (as the first in a contemplated series) the air cells, marrow spaces, labyrinthic, carotid and facial canals of a child of 4½ years.

We find that, in the child, the pyramid has been pneumatized to adult degree. There is no evidence of encroachment of mucous membrane upon marrow; the apical cells fill the tip of the bone, and almost completely surround the carotid canal; where marrow spaces lie adjacent to air cells the two do not intercommunicate, being separated by bony plates as thick as the inner table of the petrosa; the air cells, however, intercommunicate continuously and open into the tympanic cavity by several orifices, all of them situated near the point of continuity of the cavity with the auditory tube; the apical cells do not communicate with the mastoid air cells.

(4) *8. Are there 'efferent fibers' in the optic nerve of mammals?* L. B. Arey and H. V. Smith, Department of Anatomy, Northwestern University.

The existence of centrifugal fibers is an inference based solely on finding fibers within the retina that lack identifiable cells of origin. More informative, experimental evidence can now be offered:

1. All fibers disappear after retinectomy. The highest number of agryrophilic fibers found in the optic nerves of twelve dogs after 3 to 24 weeks was but 0.02% of the normal total. Osmic preparations clear slowly and are less convincing, yet, in older stages, even possible myelin rings are sparse.
2. Total fiber disappearance is not due to retrograde degeneration. To learn the possible role of retrograde degeneration, nerves were cut intracranially. The resulting decrease (as shown by silver-stained, persisting fibers and by specifically stained, degenerated fibers) was about equal in the operated and contralateral nerves. In the degeneration series, degeneration products increased closer to the operative site. Such fiber loss is apparently due to trauma and other operative sequelae. The existence of prompt, retrograde degeneration in fibers with intact cell bodies is not supported.
3. Inter-retinal fibers are not demonstrable. Following unilateral retinectomy, sparse, diffuse degenerative evidences (with a specific stain) appear in the contralateral nerve, but the amount is equalled in unoperated, control animals.
4. Conclusion. It is unlikely that an appreciable number of centrifugal fibers, either of central origin or from the opposite retina, is characteristic of the optic nerve of mammals. (See demonstration no. D5.)

(94) *9. Neurohumoralism in the embryonic heart.* Philip B. Armstrong, Department of Anatomy, Cornell University Medical College, New York City.

In this talk will be discussed the peculiar advantages of the embryonic heart as a test object for some of the concepts of neurohumoralism. The aneural heart is especially fortunate for this purpose as in its earlier stages it is completely aneural and the later innervated stages serve as excellent controls. Recent work with the neurohumors on embryonic hearts, especially acetylcholine, has almost completely ignored the concentration factor in determining whether or not the action was that characteristic of a neurohumor. Micro-cinematographs will be shown to illustrate some of the points discussed.

(49) *10. The form and relations of the premaxilla in man.* M. F. Ashley-Montagu, Departments of Anatomy, College of Dentistry and Graduate School, New York University. (Introduced by Gustav J. Noback.)

The early development of the premaxilla (intermaxilla) of the human skull has been the subject of many successful investigations within recent years, but the form and relations of this bone in the late fetal and post-natal skull has not hitherto been described. A study of some 5000 human crania, including some fifty fetal crania ranging in age from 3 to 9.27 months led to the discovery of the exact form and relations of the premaxilla in the late fetal and adult skull, and incidentally to the demonstration of the inaccuracy of the traditionally accepted description of these. It was found that by the end of the twelfth week of development the anterior sub-nasal walls of the premaxilla are completely absorbed in the substance of the maxilla. Except for the anterior nasal spine no element of the premaxilla is represented upon the facial aspect of the human skull. Moreover, it was found that practically the whole of the incisor alveoli and their septal walls are formed by the maxilla and not by the premaxilla, the posterior walls of these alveoli alone being formed by the premaxilla. (See demonstration no. D6.)

(24) *11. The human utriculo-endolymphatic valve and neighboring structures.* T. H. Bast, Department of Anatomy, University of Wisconsin.)

Histological sections of a number of ears show that the utricular duct is closed by apposition of the valve and the opposing duct wall which indicates that the valve aids in closing the utricular duct orifice. A membranous septum containing the blood vessels to the utriculus spans between the medial wall of the vestibule and the wall of the utriculus near the opening of the utricular duct. This membrane in some ears is tensed and thus by its pull on the utricular wall relaxes that segment of the utricular wall which over-lies the utricular valve. This mechanism when considered with the possibility of pressure variations between endolymph and perilymph throws some light on the probable nature of the function of the utricula-endolymphatic valve. (See demonstration no. D8.)

(10) *12. Development of lymphocytes into myelocytes in tissue culture.* William Bloom, Department of Anatomy, The University of Chicago.

Rabbits received four subcutaneous injections of extracts of Ascaris lumbricoides in Tyrode solution. Lymphocytes from the thoracic duct of these rabbits were cultured with rabbit or chicken connective tissue in rabbit plasma, cell-free rabbit bone marrow extract, and ascaris extract. After 5 days in vitro some of these lymphocytes developed into pseudoeosinophil (heterophil) myelocytes characteristic of the rabbit. On the day before the myelocyte granules appeared, the mitochondria in the lymphocytes became much smaller and more numerous as seen with Janus green. In the living cultures the specific granules were easier to detect unstained than when stained with neutral red. In the latter case it was impossible to distinguish early myelocytes from monocytoid lymphocytes. However, the separation of these two cell types was easy in the sectioned cultures stained with eosin-azure II, which showed all of the stages of development of lymphocytes into myelocytes. There was no evidence of a transformation of macrophages, fibroblasts or endothelial cells into myelocytes. Myelocytes never appeared in the control cultures of connective tissue without lymphocytes. (See demonstration no. D9.)

(111) *13. The structure of the hypertension glomerulus produced by excess pituitary tissue.* Raymond F. Blount, Department of Anatomy, University of Minnesota.

Wax models of the glomeruli of hypophysectomized, control and triple pituitary animals have been made at a magnification of 2000. These are of the glomerular mass and capsule, of the basement membrane shell, and of the endothelial core. The animals with extra pituitary tissue have a smaller glomerulus than the control in respect to the capsular space while in the hypophysectomized individuals it is larger. The glomeruli are however smaller than those of the control in both types of test animal. Just how this is correlated with the small size of the individuals is not clear. The capillary tuft in the control is more complicated than in either of the test animals. In the triple pituitary animal the number and tortuosity of the capillary loops is greatly reduced and the diameter of the vessels is decreased. On the other hand the decreased complexity in the hypophysectomized individual is associated with dilation of the vessels. In these, the space between the vessels is correspondingly reduced. The three dimensions of the models show the basement membrane to advantage. In the hypophysectomized individual it is decreased in thickness while it is greatly thickened in the triple pituitary host. A very similar thickening is found in the glomerulus of human hypertension as well as a reduced complexity of the capillary tuft.

(77) *14. Changes in the adrenal glands of spayed albino rats.* Charles M. Blumenfeld, Department of Anatomy, University of Utah.

The decrease in total weight and in water, lipin and dry matter content of the adrenal glands of rats spayed during the fourth week of life and killed at 3 or at 6 months of age has been previously reported. Further studies have shown that 2 months after spaying the decrease in size of the adrenals was due almost entirely to diminution in volume of the zona fasciculata. Compared with the glands of control rats those of the tests showed approximately the same number of mitoses in the outer fasciculate region; in the reticular zone were more degenerating cells and more partly and completely pigmented cells. Five months after spaying the marked decrease in the size of the adrenals was found due to diminution in volume of all three cortical zones, but especially the reticular. Mitoses were approximately as frequent in test as in control glands, but the reticular zone of the glands of spayed rats was distinguished by congestion near the fasciculate region, in and about which area degenerating cells and large, markedly-vacuolated pigment cells were numerous. The changes appeared primarily dependent upon the physiological age of the animals during the period of absence of the ovaries, and were suggestive, not of atrophy, but of either loss of some special function or of over-activity unaccompanied by corresponding regeneration.

(61) *15. On the cerebral cortex of Cebus.* Gerhardt von Bonin, Department of Anatomy, University of Illinois, College of Medicine.

A cytoarchitectural cortical map of Cebus was constructed from transverse sections stained with thionin. The whole surface of the isocortex of the left hemisphere was 2350 mm.^{2} The striate area covering 267 mm.^{2} as well as the somato sensory area differ little from those in man. The acoustic area is poorly differentiated. The parieto-temporo-occipital field contains well-developed para- and peristriate and temporal areas and a parietal field with indistinct boundaries. This is probably homologous to the human fields PE + PF + PG. The pre- and post-rolandic areas do not cover the operculum of the island of Reil; this bears occipitally parietal cortex and frontally a 'fronto-opercular' formation vaguely reminiscent of Economo's field FCBm. In the frontal lobe the agranular cortex takes up about half its surface. The frontal pole is covered by a granular cortex, resembling more FE than either FC or FD. The cingular gyre bears anterior agranular and posterior granular limbic areas.

Measurements of the relative breadth of the cortical layers, of cell densities and of nuclear sizes were made and analyzed statistically. They all point to a greater diversity in the brain of man than in that of Cebus.

(105) *16. Observations on the secretory granules in fixed preparations of human gastric glands.* Donald J. Bowie, Department of Histology and Embryology, McGill University, Montreal.

Methods which had previously been employed for studying the pepsinogen granules in the gastric glands of laboratory animals were applied to specimens of human gastric mucosa obtained at operation. In the human the pepsinogen granules in the body chief cells are preserved in a manner similar to that obtained in laboratory animals. In addition the secretory granules in the mucous neck cells are well preserved and stained. Both the body chief cells and the mucous neck cells may have very irregular outlines. In the region where there is a change from mucous to body chief cells, certain cells appear to exhibit some of the characteristics of each of the two types. (See demonstration no. D12.)

(99) *17. The innervation of the teeth.* Alton D. Brashear, Department of Anatomy, Louisiana State University Medical Center. (Introduced by T. W. Todd.)

An analysis of nerve fiber components involved in the selective distribution of sensation from the teeth and surrounding tissues is presented. Branches of the trigeminal nerve supplying the teeth of the cat contain myelinated nerve fibers of all sizes as well as unmyelinated fibers. Pulps of human and cats' teeth contain a majority of small myelinated nerve fibers less than 6 μ with the largest fibers measuring 9 μ. The peridental tissues surrounding these teeth contain nerve fibers of all sizes with the largest myelinated fiber measuring over 14 μ. In some branches of the dental plexuses of the cat there is a segregation of unmyelinated and small myelinated nerve fibers. Other branches, however, contain a greater percentage of larger myelinated nerves. The experimental evidence offered indicates that pulpal nerves are predominantly the peripheral processes of small or medium size nerve cells in the gasserian ganglion of the cat.

Further histological observations on human teeth indicate that the nerves of the pulp end freely about the odontoblasts, and between these cells and the dentin.

From our general knowledge of the functions of peripheral nerves and from evidence presented in this paper it is suggested that pulps of teeth are concerned mainly with painful sensations. The organ of touch for the tooth probably is located in the peridental tissues.

·(93) *18. Effect of blood streams on the developing heart.* J. L. Bremer, Department of Anatomy, Harvard Medical School.

In chick, the heart septa, with exception of the interatrial septum, form between two blood streams, from right and left vitelline veins, respectively. The left vein mouth is depressed by the coiling of the heart, the ventricular region impinging upon and lifting the right side of the common atrium. This is true even in heterotaxic chicks where reverse relations are primarily present. The left stream is violently deflected by the beat of the rounded right atrial wall (where the blood runs dorsally) and passes diagonally along the atrial roof to the left atrial wall, morphologically more cranial, whence the peristaltic beat forces it down the left side of the atrioventricular canal. It thus pursues a spiral course around the right stream. The endocardial cushions form between the two streams, and after their establishment the relations causing the spiral streams are lost.

Manipulation of parallel rubber tubes, first wound in a similar spiral and then forced to coil like the heart, shows a reversed spiral in the bulb. This is seen in cast models of chick hearts. A septum, primarily transverse between fourth and pulmonary arches, follows this bulbar spiral caudally to meet the endocardial cushions. The ventricles are separate pouches at the points of sharpest curvature of the two streams below the atrioventricular canal. The streams are shown in moving pictures.

(68) *19. The course of the secondary vestibular and related fibers in cats as shown by their degeneration following lesions of the vestibular nuclei and cerebellum.* A. R. Buchanan, Institute of Neurology, Northwestern University Medical School. (Introduced by S. W. Ranson.)

Lesions of the vestibular nuclei were produced in a large number of cats by means of a Horsley-Clarke stereotaxic instrument. After allowing 11 to 15 days for degeneration to occur, the animals were killed, the brains and spinal cords were removed and were fixed by a modified Marchi technique recommended by Swank. The course of the secondary vestibular fibers as determined by study of the degeneration was as follows: Axons of cells in Deiters' nuclei course rostrally in the contralateral medial longitudinal fasciculus and in the extreme lateral horn of the homolateral fasciculus (ascending tract of Deiters); other axons from the same group of cells descend in both medial longitudinal fasciculi and in the homolateral vestibulospinal tract; the medial nuclei give rise to fibers which course caudally and rostrally in both the contralateral and homolateral medial longitudinal fasciculi; Bechterew's nuclei contribute fibers to the homolateral ascending medial longitudinal fasciculus and to the homolateral vestibulospinal tract; the descending nuclei give origin to fibers which descend in the medial longitudinal fasciculi of both sides. Concomitant injury of cells in the cerebellar nuclei resulted in degeneration of the brachium conjunctivum and fasciculus uncinatus.

(76) *20. A preliminary study of the effect of cortical adrenal extract on sympathetic ganglia of adrenalectomized cats.* B. I. Burns, Department of Anatomy, Louisiana State University Medical Center.

The author, in collaboration with J. D. Reese and A. H. Sellmann, has shown degenerative changes in the sympathetic ganglia in adrenalectomized cats. The present report is based on a study of sympathetic ganglia in adrenalectomized animals to which Eschatin (Parke, Davis preparation of cortical adrenal extract) was administered subcutaneously for a time corresponding to the usual survival period of adrenalectomized animals which were untreated. Material from four cats demonstrates that Eschatin inhibits the ganglion cell changes resulting from adrenalectomy and indicates that deprivation of this substance is responsible, directly or indirectly, for the cell alterations described.

(125) *21. The differentiation of associated gonad primordia from different rat embryos studied in kidney grafts.* Adrian Buyse, Department of Zoölogy, The University of Rochester and Osborn Zoölogical Laboratory, Yale University.

Buyse ('35, '36) has shown that sex hormones of the adult are ineffective in modifying differentiation of rat gonad primordia when transplanted to the kidneys of sexually mature and gonadectomized hosts of both sexes. Consequently a series of grafts of combinations of singly isolated primordia derived from different embryos of 12, 13 and 14 days gestation and combinations of 12- and 14-day primordia were transplanted in pairs to the kidneys of sexually mature and gonadectomized hosts of both sexes to determine what influence the sex of the associated gonad might have on the capacity of the rudiments to differentiate. Eighty-seven out of ninety-five such combinations developed as grafts. There are ♂♂ 19; ♂♀ 47, of which twenty-one are grafts of testes combined with areas of rete cords and twenty-six testes in combination with no rete cords; ♀♀ 21, consisting of two sets of rete cords in twelve cases and only one set of cords in nine cases. Ovarian development is inhibited more strongly when isolated primordia are transplanted for no ovaries comparable in degree of differentiation to those reported previously have been recovered in this series. On the basis of expectancy rete cord areas have been assumed to represent ovaries. Adverse mechanical conditions in the graft affect the differentiation of individual zygotically determined female primordia more unfavorably than the differentiation of the mesonephric-gonad complex as transplanted heretofore.

(28) *22. Studies in the dynamics of histogenesis XVII. Biophysical experiments on the hydro-capillary wave mechanics of gradient internal protoplasmic colloidal compression during ameboid, muscular motions and nervous impulse conductions.* Eben J. Carey, Department of Anatomy, Marquette University School of Medicine.

Differential bombardment by water molecules activates and inhibits amebic colloidal granular motions. Oscillating colloidal particles emanate condensation-rarefaction waves: internal protoplasmic colloidal compression. Reversible localized high, low protoplasmic pressures cause reversible aggregation, cataphoresis, streaming, changes of shape, viscosity. Tension multiplies, aligns protoplasmic-pressure waves or cross striations within capillary tubes, naturally, in striped muscular, nervous tubular fibers, experimentally, in living amebae. Confining living amebae, within capillary glass tubes, fifty, 80 μ lumen diameter orients colloidal granules into longitudinal and transverse rows. Alternate transverse dark, light bands multiply; shift positions during conduction, variable amebic pressure waves. Amplifications by longitudinally directed, capillary pressure radiations from uniformly high-frequency oscillating colloidal granules cause amebic 'explosions.'

Longitudinally stretching leeches with 5-gm. weights cause rhythmic contractions, five to 120 per minute, which may continue for 7 days. Histologic transformation occurs, smooth, fusiform, tubular muscles into cross striated types. Hydraulic, capillary tubular, ventral nerve cords have, likewise, short alternate dark, light bands: high-frequency pressure wave reactions to increased tensional load. Adequate rhythmic longitudinal tensional stimuli multiply transverse, reciprocal, alternating action of internal potential, kinetic pressure wave energy, expressed as cross striations.

Strychnine, spasticity, electricity, heat, pressure multiply muscular, nervous pressure waves, cross striations. Histological expressions, capillary colloidal nerve-pressure waves are: Nissl colloidal concentric whorls; axone back pressure hillock, dilatations, constrictions, striae, funnels, rings; myelin incisures, segmentations. (See demonstration no. D14.)

(26) *23. Development of autonomic innervation in the fetal iris of the pig.* Kermit Christensen, Department of Microanatomy, St. Louis University School of Medicine.

Irises of pig fetuses prepared by silver methods have been studied to determine the time when the intrinsic musculature becomes innervated. In fetuses of 100 to 150 mm. peripheral nerve arches have been formed in the iris from which fibers grow radially. In fetuses of 150 to 250 mm. in which the intrinsic muscles are more differentiated a close structural relationship appears between the nerve terminations and the smooth muscle. The time of appearance of nerves in the iris is correlated with the activity of the iris in response to treatment by a choline compound.

(116) *24. Quantitative effects of theelin on body growth and endocrine glands of young albino rats.* Fred W. Clausen and Clay B. Freudenberger, Department of Anatomy, University of Utah Medical School.

Twenty-five female Wistar albino rats were injected daily with 200 international units of theelin in oil for a period of 10 days. The rats were started on the experiment at 21 days of age and were autopsied on the thirty-first day of age. Twenty-five litter-mate controls were injected with olive oil.

The body weights, body lengths, and tail lengths were studied as well as the weights of the following endocrine organs: hypophysis, thyroid, thymus, suprarenal glands, and ovaries. Biometrical methods were employed in evaluating the results.

The hypophysis and thyroid were significantly heavier in the test group. The body weight and thymus were significantly smaller in the tests. The ovaries were heavier, and the suprarenal glands were lighter in the tests than in the controls. The body and tail lengths were less in the tests than in the controls. All of these latter differences were of doubtful significance.

The most marked difference between the two groups of animals was that found in the thymus gland. The significance ratio for this difference was 13.1.

(14) *25. Cyclic changes in lymphatic tissue.* Eleanor A. Conway, Department of Anatomy, The University of Chicago. (Introduced by William Bloom.)

The lymphatic tissue of rabbits and guinea pigs infected with B. monocytogenes passes through stages of hyperplasia and depletion. In the initial hyperplasia and in that during recovery from depletion, new foci of intense proliferation of lymphocytes arise in any part of the diffuse or nodular lymphatic tissue. In diffuse lymphatic tissue these new foci are new lymphatic nodules. A new focus developing within a preexisting nodule forms the structure usually described as a primary nodule with a secondary nodule within it. The structure of the nodule varies with the stage of its development and the stimuli acting on it. Accordingly, lymphatic nodules are variable in their situation and their cellular constitution. (See demonstration no. D17.)

(92) *26. Correlation in the structural and functional development of the heart as studied by heteroplastic grafting.* W. M. Copenhaver, Department of Anatomy, College of Physicians and Surgeons, Columbia University.

The earliest contractions in the heart of Amblystoma punctatum embryos (Harrison's stage 34), arise in the ventricular region. By stage 35, the atrium initiates the beat and dominates the rhythm until the sinus venosus begins to function (stage 39). By transecting the heart at different levels, the intrinsic rhythm of all parts (including bulbus cordis and truncus arteriosus), has been studied at the various developmental stages.

Development occurs more rapidly in A. tigrinum than in A. punctatum although the latter has a higher pulsation rate. When hearts are transplanted between these species, either to normal or heterotopic positions, the donor pulsation rate is retained.

Sections of transplanted and control hearts have been studied for striations, yolk droplets, fats and glycogen. Whole mounts of the heart treated with Zenker-osmic acid and sections of similarly fixed embryos failed to show striations when pulsations begin. Striations in the transplant differentiate at the donor control rate. Yolk and fat droplets vary in number with the developmental stages and differ in the two species. In transplanted hearts, the yolk droplets are 'resorbed' at the donor rate. Differentiation of other structures of the graft, such as ventricular trabeculae, also show the influence of an inherent tissue rate of development.

(129) *27. Some genetic implications in dissecting room material.* C. H. Danforth, Department of Anatomy, Stanford University.

The extensive studies of a generation or more ago on anatomical variations reached a peak of interest during the active period of such men as Gruber, Macalister, Wiedersheim, Schwalbe and Pfitzner, before ideas of genetics had become known and at a time when prevailing ideas of homology endowed morphological structures with a sanctity not always accorded them today. When the range of variations had been canvassed and their statistical frequencies investigated, there ensued a period of diminishing returns for investigators in this field. But with gradual change in points of emphasis, which came about largely through increased interest in genetics, another cycle has been completed and anatomical variations again occupy a place of interest. This is reflected in the current literature, particularly that of Germany. The problem now is not merely the incidence of variations but their interpretation in terms of developmental genetics. Certain phases of this problem can be studied to advantage in dissecting room material, and it is this aspect of the subject which it is hoped to discuss.

(74) *28. The veins of the endometrium (Macacus rhesus) as a source of the menstrual blood.* Garman H. Daron, Departments of Anatomy, The University of Chicago and New York University College of Medicine. (Introduced by Bertram G. Smith.)

The venous pattern of the endometrium consists of many small veins coursing parallel to each other in a centrifugal fashion from the uterine lumen without uniting to form larger trunks basalward. Anastomoses at acute angles between these parallel veins are very numerous. In the middle third of the endometrium there are large 'venous lakes' which are greatly dilated veins at sites of certain anastomoses. This general venous pattern is perhaps a result of the characteristic arterial distribution, and the periodic loss and regeneration of the superficial zone of the endometrium.

By an intravenous injection, in vivo, and almost instantaneous fixation, the site of bleeding during the injection is demonstrable. Evidence of bleeding from the ends of coiled arteries or their branches in the denuded endometrium is rarely detected; however, bleeding veins are readily found. The veins most freely bleeding are those that course direct from the 'venous lakes' without intervening anastomoses. This is proof that menstrual blood is lost by venous regurgitation from a source deep in the endometrium. Observations here indicate that a considerable amount of the menstrual blood is lost in this fashion subsequent to the initial rupture of the subepithelial hematomata and general denudation.

It is suggested that while the initiation of menstruation is perhaps primarily an involvement of the arteries, the control of the total amount of blood lost is a phenomenon implicating principally the veins.

(34) *29. The ratio of myelinated to unmyelinated fibers in regenerated sciatic nerves of monkey.* H. A. Davenport, Herman Chor, Ralph E. Dolkart, Departments of Anatomy, Nervous and Mental Disease, and Chemistry, Northwestern University.

Six months after cutting and suturing the right sciatic nerve in eight Macacus rhesus monkeys, the animals were killed and both the regenerated and normal nerves removed for histologic study. Data obtained consist of estimates of the number of myelinated (by OsO_4) fibers and total axons (by Ag) in the following locations: 1) 3 to 5 mm. proximal to the lesion, 2) 3 to 5 mm. distal to the lesion and 3) from a level of the normal nerve similar to that of the lesion. The results indicate: 1) that there were 91,000 to 119,000 axis cylinders in the normal nerves, 2) that the number increased to 105,000 to 142,000 above the lesion and 3) that 76,000 to 102,000 grew through, i.e., were found on the distal side. Myelinated fibers ranged from 30,000 to 39,500 in the normal nerves, from 32,000 to 43,000 above the lesion, and from 18,000 to 36,000 below the lesion. We conclude that no very consistent nor marked change in the myelinated to unmyelinated ratio has occurred in the regenerated nerves studied at the end of 6 months.

(107) *30. An analysis of volume changes in tissues subjected to microscopical technique.* W. T. Dempster, Department of Anatomy, University of Michigan.

From fixation to sectioning and mounting, tissue volume is altered by the various reagents used and in different ways. Depending on the osmotic pressure of tissues, there is characteristic swelling or shrinkage which is largely irrespective of the fixatives employed. Acetic acid and formalin as simple fixatives caused tissue swelling in the tissues studied while the six other most common reagents caused shrinkage of characteristic rate and extent. The assumption that fixing agents can be so mixed that shrinkage caused by one agent may be balanced by swelling caused by another is erroneous. The component reagents in a fixing mixture act in sequence according to their individual rates of penetration. Depending on the sequence of action of reagents, a specific amount and rate of tissue shrinkage is induced, and the degree of shrinkage cannot be predicted on the basis of action of the simple agents alone. Mixtures containing acetic acid or formalin may produce greater shrinkage than similar mixtures without them.

Following various fixatives, dehydrating agents cause varying degrees of shrinkage. In alcohol dehydration, 70% and higher grades of alcohol are responsible for shrinkage. Clearing and embedding agents cause different amounts of shrinkage following different fixatives. Paraffin in changing from the liquid to the solid state shrinks 10 or more per cent. In cutting paraffin, sections are compressed so that thin sections are markedly distorted. Compression is irrespective of paraffin melting point.

(40) *31. Further observations on the action of pituitary hebin upon the reproductive system of the chick embryo.* L. V. Domm and E. A. Dennis, Department of Zoölogy, The University of Chicago.

Incubating eggs, from brown Leghorns and a hybrid variety with sex-linked plumage, received single and repeated injections of pituitary hebin. Injections which varied from one to six in number were made at varying ages, daily or on alternate days, the earliest began at 48 hours and majority terminated on ninth day. In a few experiments injections began on twelfth day and terminated on sixteenth. Surviving embryos were killed and examined on eighteenth and a few on nineteenth day. Some were allowed to hatch.

Examination revealed hypertrophy of gonads especially evident with higher dosage. The degree of hypertrophy varied and was more general and pronounced in females than males. Particularly significant was the striking hypertrophy of rudimentary right gonad which frequently surpassed the normal left in size, rather significant considering the lack of response in our post-embryonic injections. Sections of hypertrophied ovaries invariably show a striking hypertrophy of medullary layer with very little or no hypertrophy of cortical layer. Cortical layer is usually low, irregular, and frequently discontinuous, hence greater size of treated ovaries is due to medullary hypertrophy. The composition of the hypertrophied rudimentary right gonad in majority of cases is entirely medulla. In some cases small patches of surface cortex are found.

Comparing results in the two varieties we found hybrids not only more viable, but the gonads in both sexes were also more responsive to treatment. than the Leghorn.

(120) *32. The effects of theelin on hypertrophy and regeneration of the ovaries.* Frederick E. Emery, Department of Physiology, University of Buffalo.

The effects of theelin on the estrous cycle, ovaries, hypophysis, and several other organs have been studied in semi-ovariectomized rats, in rats with one-half of each ovary removed, and in rats with entire ovaries. Theelin in 1 to 20 rat units daily for 6 weeks had little, if any, effect on the secretion of the hypophysis as shown by 1) normal hypertrophy of the remaining ovary, 2) normal regeneration of ovarian fragments, and 3) insignificant reduction in the weight of the entire ovaries. The length of the estrous cycles was practically unaffected, but the per cent of time in estrus was somewhat longer in the theelin injected groups. This latter effect may not be specific as shown by similar results in the saline injected controls. The results indicate that ovarian hypertrophy and regeneration may not be entirely controlled by the action of theelin on the hypophysis.

(20) *33. The effect of the destruction of the germinal crescent on the origin of the germ cells and the development of the gonads in the domestic fowl.* J. M. Essenberg and Arthur J. Svejda, Department of Anatomy, Loyola University School of Medicine, Chicago. (Introduced by R. M. Strong.)

Three methods of operation of the embryo were used: electrical cauterization, excision of parts of the blastoderm, and incision into definite areas of the blastoderm. The areas destroyed were: the germinal crescent and corresponding parts caudally and laterally to the embryo. Controls were kept for all experiments. Two age groups were selected: 18 to 24 and 25 to 50 hours of incubation. In the cauterization experiments it was desired to ascertain the significance of the crescent cells or the so-called primordial germ cells on the formation of the embryo including definitive germ cells and the gonads. The excision experiments were to determine the probable heat or chemical effect of cauterization on development. In the incision method the cellular contents of the operated region remained practically uninjured, only the vascular connections being severed. The results tend to prove that the mortality of the embryos is related to the intensity and extent of injury rather than to the area injured. The survival and development of the embryo depends upon the vitality of the embryo to reconstruct an efficient vitelline circulation after the injury. The total number of eggs used was 948.

(44) *34. Effect of interstitial cell stimulating hormone on hypophysectomized male rats.* Herbert M. Evans, Miriam E. Simpson and Richard I. Pencharz, Department of Anatomy and Institute of Experimental Biology, University of California. (To be presented by Miriam E. Simpson.)

Report was previously made of the separation of sheep pituitary extracts with ammonium sulfate into follicle stimulating, luteinizing and interstitial cell stimulating fractions as tested in hypophysectomized males and females. As regards the male, it was shown that some of the effects previously attributed to the action of the luteinizing hormone were due to interstitial cell stimulating hormone which repaired Leydig tissue and thereby stimulated the accessories. The effects were clearly expressed in 3 days. The present communication reports similar experiments of 10 and 25 days' duration. The previously reported effects became somewhat more pronounced in the 10-day experiments but in some animals regression had begun by the twenty-fifth day.

The effects of the extracts cannot be attributed to contamination of the interstitial cell stimulating preparations with adreno-corticotropic hormone, for the adrenals were not enlarged and furthermore the effects were secured with hypophysectomized and doubly adrenalectomized males.

(109) *35. The significance of pigment (melanin) formation and destruction in cells as studied by means of oxidation-reduction indicator dyes.* Frank H. J. Figge, Department of Anatomy, University of Maryland Medical School.

In 1932, Margaret R. Lewis reported that some of the indophenol dyes destroyed the pigment in tadpoles. Last year it was shown that: 1) the effectiveness of the dyes in eliminating pigment in different species of Amphibia paralleled the metabolic rate; 2) they were most effective in Necturus, a form with a low metabolism and resembling the relatively non-pigmented cave salamanders; 3) they eliminated melanin in hypophysectomized animals; 4) the rate of dye decolorization bore no relation to the rate of pigment destruction.

This year it will be shown that: 1) the action of the dye is inhibited by cold; is not photodynamic; is not effective in dead animals, in pigment removed from cells, in large animals, nor when injected intraperitoneally; 2) the dyes do not destroy dopa-oxidase, the enzyme necessary for pigment production.

The tentative hypothesis satisfying these observations is: The formation and presence of melanin in a cell is dependent not only on the presence of enzymes (tyrosinase and dopa-oxidase) in the cell, but also on the maintenance of a certain oxidation-reduction potential within the cell. The indophenol dyes are capable of shifting or re-poising this intracellular oxidation-reduction potential, causing pigment to disappear in cells which normally manufacture and store pigment, while pigment appears and accumulates in cells which usually do not contain much pigment.

(32) *36. The jugular fibers in the vagus nerve of the cat.* James O. Foley and Franklin S. DuBois, Department of Anatomy, University of Alabama.

In a previous report (DuBois and Foley, 1936 meeting) based on studies made by the chromatolytic method, it was suggested that relatively few of the fibers with cell bodies in the jugular ganglion descend in the cervical trunk of the vagus and that the majority are distributed via the auricular nerve. Additional evidence in support of this view is submitted.

The vagus nerve was sectioned and subsequently the nodose ganglion was removed in six cats. It was hoped that following such procedures the number of cells in the jugular ganglion might be compared with the number of peripheral processes but regeneration in the segment of vagus distal to the ganglion and technical difficulties made some of the material unsatisfactory for numerical analysis. In one animal, there were 8451 cells in the jugular ganglion, 6237 fibers in the auricular nerve and 1298 fibers in the vagal stump. In another, the auricular nerve could not be analyzed but in the vagus 4105 axons were counted; however, some of these may have been the products of regeneration. Although enumeration of the axons in the remaining vagus nerves was impossible, osmicated preparations demonstrated 324, 350, 456 and 633 myelin sheaths, respectively. Additional auricular nerves (some from the above animals, others previously prepared) were studied. The axon counts were 6533, 8154, 9121, 9371 and 10928, respectively. (See demonstration nos. D20 and D22.)

(119) *37. The effect of prolonged injections of oestrone (Progynon) in the immature alligator, with particular reference to the Müllerian ducts.* Thomas R. Forbes, Department of Anatomy, The University of Rochester School of Medicine and Dentistry. (Introduced by R. K. Burns, Jr.)

Injections begun when the animals were 15 months old were made into both males and females during an 80-day period. Each individual received a total of 9300 rat units of Progynon. At autopsy the oviducts of the injected females were found to be enormously hypertrophied, having increased approximately ten times in length and eight times in diameter as compared to the controls. The particularly rapid growth of the oviducal mucosa had caused it to be thrown into extensive folds and branched gland-like out-growths. Although it is difficult to find vestiges of a Müllerian duct in control males of this age, the injected males had developed short, intermittent segments of Müllerian duct at the level of the testis. A fold of hyperplastic connective tissue lying between the ends of the duct segments indicated at least a limited response of tissue in a region corresponding to that in which the oviduct occurs in the female. The wolffian ducts of the injected males and females were not visibly affected.

(115) *38. The effect of continued theelin injections on the body growth and organ weights of young female rats.* Clay B. Freudenberger and Fred W. Clausen, Department of Anatomy, University of Utah Medical School.

Twenty-five female Wistar albino rats were injected subcutaneously with 200 international units of theelin in oil every other day from the twenty-first day of life to about the hundredth day of life. Twenty-five controls were injected with olive oil. At the end of the experimental period all animals were killed, completely autopsied, and the organs weighed. Modern biometrical methods were used in interpreting the results.

The average body weight of the theelin-injected rats was definitely less than that of the control group. On the fourth day the average weight of the test group was less than the control group. The difference became significant on the fourteenth day of experimentation and continued as such to the end. At autopsy the average body weight of the theelin-treated rats was 80% of that of the controls. The test animals were normal, except for smaller size.

The average body and tail lengths were significantly smaller in the test rats. The following parts and organs were found to be significantly smaller in the test rats: head, integument, humerus, femur, hypophysis, thyroid, thymus, brain, spinal cord, eyeballs, lungs, submaxillary glands, alimentary group, stomach, spleen, kidneys, ovaries, and uterus. The lengths of the humerus and femur were significantly less in the test animals. The suprarenal glands, heart, and intestines were smaller in tests, but not significantly. The liver showed no change.

(38) *39. Cytological studies of the anterior pituitary of the rabbit before and after its physiological stimulation.* H. B. Friedgood and Alden B. Dawson, Department of Physiology, Harvard Medical School and the Biological Laboratories, Harvard University. (To be presented by Doctor Dawson.)

Rabbits generally ovulate 10 to 12 hours post coitum. Hypophysectomy, if done within 30 minutes after mating, prevents ovulation. Ovulation proceeds normally if hypophysectomy is delayed 90 minutes. This indicates that enough gonadotropic substance to induce ovulation is secreted by the anterior pituitary within 30 to 90 minutes after mating. The anterior pituitary was studied at frequent intervals after mating.

The pituitaries of the control and experimental groups were fixed in formalin or formalin and corrosive sublimate. Stained by a modification of Mallory's azan method, the usual three classes of cells are demonstrated. Another granular cell, hitherto unrecognized, is selectively stained with azo-carmine. It becomes large and contains coarse, highly refractive granules. In normal control animals (sacrificed in oestrus without mating or immediately after mating) these carmine cells occur singly or in scattered groups and there is considerable variation in the number present.

An increase in the number of carmine cells occurs within 45 minutes after mating. They dominate the cytological picture from 1½ to 4 hours, occurring in large irregular patches without apparent pattern of distribution. Their number then gradually decreases until the reaction becomes inconspicuous at 12 to 15 hours. Although not completely studied quantitatively the preliminary observations indicate that this cytological reaction occurs coincidentally with the period during which the gonadotropic hormones are released from the anterior pituitary. (See demonstration no. D23.)

(81) *40. The adrenal-pituitary relationship in lactation.* Robert Gaunt and Warren O. Nelson, Department of Biology, Washington Square College, New York University, and Department of Anatomy, Wayne University Medical School.

Purified pituitary lactogenic hormone, unlike crude pituitary extract, will not induce lactation in hypophysectomized guinea pigs. It will do so if adrenal cortical hormone is simultaneously administered (Nelson and Gaunt; Gomez and Turner).

In the present work, hypophysectomized guinea pigs given purified lactogenic extract plus either adrenotropic hormone (Collip) or high doses of NaCl lactated. This suggests a functional separability of adrenotropic and lactogenic hormones.

In lactating, post-partum guinea pigs, production of 'artificial adrenal insufficiency' by injection and subsequent withdrawal of intraperitoneal isotonic glucose solution either partially or completely inhibits lactation.

Adrenalectomized pigs, given either crude pituitary extract or purified lactogenic preparations with cortical hormone, lactated within 24 to 60 hours. The same result is obtained if a fragment of adrenal, even insufficient to maintain life, is left intact.

In completely adrenalectomized guinea pigs, neither crude nor purified pituitary extracts alone induced lactation. The survival time of animals thus treated ranged from 3 to 12 days after initiation of lactogenic treatment. The failure of purified extract is more clearly indicated than the crude extract, because with the former longer survival was obtained. Although these animals were dying of adrenal insufficiency, and for that reason might not have been expected to lactate, it is noteworthy that cases dying from the effects of hypophysectomy or other causes can be made to lactate when properly treated.

(45) *41. The relation of the spleen to the development of the refractoriness to gonadotropic substances in the rat.* Albert S. Gordon, William Kleinberg and Harry A. Charipper, Department of Biology, Washington Square College, New York University.

Young immature (40 to 50 gm.) hooded female rats respond to daily 10 R.U. injections of prolan primarily by an increase in ovary size which reaches a maximum approximately 10 to 15 days after beginning of treatment and then regresses, becoming normal or subnormal in size within 2 to 3 months despite continued injections. The ovaries of splenectomized litter mates of these animals, however, subjected to the same treatment continue to grow, attaining weights approximately 2 to 3½ times that of the controls 20 to 30 days after splenectomy. This increase in weight is due almost entirely to an increase in the size, and in some cases, in the numbers of corpora lutea. With continued treatment the ovaries of the splenectomized animals begin to regress after about 30 days due, we believe, to the establishment of a compensatory mechanism similar to the spleen.

These experiments point to the spleen as a source of the anti-gonadotropic principle, and which we interpret as another manifestation of reticulo-endothelial activity.

It is of prime importance that the animals be completely free of bacterial infection (especially Bartonella muris in the rat), since this is necessarily a factor affecting reticulo-endothelial activity. Experiments of a similar nature are now being conducted with the thyreotropic principle and tests being made of the inhibitory effects of serum from normal and splenectomized animals injected with various endocrine principles.

(90) *42. Early development of the rat heart in vitro.* Charles M. Goss, Department of Anatomy, Columbia University.

Rat embryos removed from the uterus during the tenth day were cultured in hanging drops. The explant consisted of the intact embryo, amnion, yolk sac and ectoplacental cone. Embryos with cardiac plates continued development until the two lateral primordia had fused into a single saccular ventricle and the atrium had appeared. The earliest contractions were observed in a few cells of the lateral wall of the left ventricular primordium before the formation of a unified ventricle. They were seen with a magnification of 500 diameters; at 37°C. they were surprisingly regular, with an average rate of 42 per minute. Intermittance and other irregularities were usually attributable to environmental changes, to which the embryos were exceedingly susceptible. After the ventricle had become a saccular organ, both sides contracted regularly at an average rate of 48. In some instances the right and left portions followed independent rhythms at slightly different rates. After the myocardium on the atrial side of the primitive A-V groove became active, the rate soon quickened to an average rate of 82. The ventricular contraction spread from this region in a peristaltoid wave which travelled from left to right rather than from venous to arterial ends of the ventricle. In some cases observed for the first time after the atrium had become active, the wave travelled from right to left.

(118) *43. Effect of estrone on the oviducts of Triturus viridescens.* Ronald Grant, Department of Zoölogy, Columbia University. (Introduced by William Etkin.)

An estrogenic substance can be extracted from amphibian ovaries but injection of preparations containing estrone into female Triturus viridescens did not cause hypertrophy of the oviducts. Intraperitoneal injections of oily and aqueous solutions of the hormone were given to immature, mature and castrated females for periods of up to 12 weeks and quantities up to 6000 I.U. were administered. Ovarian atrophy followed prolonged injection of oily preparations.

(86) *44. The dual nature of anterior pituitary influence on the testis.* Roy O. Greep, Harvard University. (Introduced by P. E. Smith.)

The anterior lobe of the hypophysis exerts its control over the structural development and functional activity of the male gonad by means of two hormonal substances. Experimental evidence for this belief was obtained from a study of the action of two pituitary fractions, termed FSH and LH, in the male. The differential action of these fractions was most clearly demonstrated in hypophysectomized male rats where the induced changes are emphasized by being built upon an atrophic pattern. Here the FSH caused the seminiferous epithelium to undergo marked proliferative activity but did not restore the interstitial cells and as a consequence the secondary sexual structures remained atrophic. The LH, conversely, failed to alter the atrophic condition of the tubular epithelium but did bring about marked repair and hypertrophy of the interstitial cells associated invariably with enlargement of the accessory sexual organs in a causal relationship. The evidence for a possible general application of the dual concept of pituitary gonadotropic function in the male has been strengthened and extended by data obtained from a study of mammals (rat, monkey, ground squirrel and guinea pig), birds and amphibians.

(126) *45. The relation of the developing apocrine sweat glands to the maturation of the reproductive system in children.* William Greulich, Department of Anatomy, Yale University School of Medicine.

The apocrine sweat glands of the axilla are said to undergo cyclic variations in secretory activity during the menstrual cycle.

The purpose of the present study was to determine the time at which apocrine sweat glands begin to function in children, and to attempt to relate it to the concomitant developmental status of the reproductive organs.

Post-mortem material was obtained from fifty-nine children, thirty-four boys and twenty-five girls, ranging in age from 8 to 17 years. The degree of development of the apocrine sweat glands of the axilla and mons pubis was compared with that of the testes and in some cases also of the prostate, in boys, and with that of the vagina, uterus, and ovaries in girls. The absence or degree of differentiation of pubic and axillary hair was noted in each instance. In addition, in older girls, the secretory phase of the apocrine sweat glands was checked against the stage of the menstrual cycle as indicated by the condition of the uterine mucosa.

(36) *46. The innervation of the hypophysis in the cat.* Gene W. Hair, Department of Anatomy, University of Buffalo. (Introduced by W. J. Atwell.)

In the cat the sympathetic innervation of the anterior pituitary is from the carotid plexus, as Dandy has reported for the dog. The majority of the fibers arise from this plexus and accompany the larger superior hypophyseal vessels which pass directly to the anterior lobe; other fibers from the plexus accompany smaller vascular channels through the pars tuberalis to the anterior lobe. These sympathetic fibers terminate in knob-like endings against the gland cells.

Fibers having origin from the supra-optico-hypophyseal tract pass through the marginal zone of the infundibular stalk to enter the pars tuberalis. Some of these terminate in the pars tuberalis while others become intimately associated with those fibers of sympathetic origin which are passing through the pars tuberalis to enter the anterior lobe. The possibility of a termination of fibers of hypothalamic origin in the anterior lobe is discussed.

Some of the fibers of the supra-optico-hypophyseal tract which enter the pars nervosa possess spiral endings covered by a protoplasmic sheath. Numerous fibers from the same source enter the pars intermedia where they terminate with enlarged endings both on blood vessels and in relation to epithelial cells.

(5) *47. The development of transplanted limb buds of chick embryos and their effects upon the central nervous system.* Viktor Hamburger, Department of Zoölogy, Washington University, St. Louis. (Introduced by R. J. Terry.)

Wing and hind limb primordia of chick embryos incubated for 52 to 75 hours were transplanted to the flank of embryos of the same stages. In 40% of the surviving cases, the transplants had developed normally and had attained normal or almost normal size. In five out of twenty-four cases fixed 5 to 6 days after operation no nerves had entered the transplant. In the other cases, nerves of variable size, either branches from the brachial or from the lumbo-sacral plexus or trunk nerves, had supplied the transplant. Morphogenetic and histological differentiation was equally normal in nerveless, in poorly innervated and in well-innervated transplants.

Spontaneous movements were observed in those transplants which were at least partly supplied by limb nerves.

The spinal ganglia of nerves innervating transplants reacted to even small increases in their peripheral fields by hyperplastic growth. Hyperplasia of the lateral motor horn of the spinal cord was definitely established in six cases by counting and comparing the number of the large lateral motor horn cells of the over-loaded and of the normal side. Cases in which the brachial region had supplied the transplant were, for technical reasons, particularly favorable for such studies. They showed that the hyperplastic effect (20% and more) was strictly localized at that part of the lateral motor column which actually gave rise to nerves supplying the transplant.

(122) *48. Masculinizing effect of male hormone substance upon female reproductive tract.* James B. Hamilton, Departments of Anatomy and Physiology, Albany Medical College. (Introduced by E. Allen.)

Masculine changes were produced in the female reproductive tract and in the endocrine glands of fifty rats, nine rabbits, nine puppies and five monkeys after administration of male hormone substance. The clitoris assumed a large penile form in puppies and in monkeys, projecting beyond the frenulum. Erections could be elicited. The preputial glands and Skene's ducts, homologous to structures in the male reproductive tract which are controlled by the male hormone, responded vigorously to male hormone but were not stimulated by oestrone. In general, the action of the male hormone on the female animal was masculine in organs which are well developed in the male by the male hormone. The response of uterus, vagina, adrenal and pituitary was but slightly oestrogenic, predominantly an 'androgenic' effect, peculiar to male hormone substance.

(30) *49. The effects of central and peripheral lesions on spinal ganglion cells.* Kendrick Hare and Joseph C. Hinsey, Department of Physiology, Cornell University Medical College, New York City.

Cutting the dorsal roots proximal to the ganglia causes no changes in the neurons within the ganglia but distal nerve section produces chromatolysis in some of the ganglion cells. To determine if distance from the cell body and time between operation and autopsy were the factors responsible for the differences in the reaction of neurons to central and peripheral lesions, dorsal roots of L7, S1 and 2 were cut near the cord on the left side and the right sciatic nerve cut above the neck of the femur. Thus the central and peripheral lesions were approximately equidistant from the ganglion, a fact established at autopsy. Fourteen operated cats were allowed to live either 6, 8, 10, 12, 14, 21, 28 or 35 days. Corresponding ganglia from the right and left sides were mounted and stained on the same slide. No alterations in the ganglion cells were detected following severance of their central processes and only moderate reactions involving less than half the cells after transection of the sciatic. Large and small cells were affected. Absence of chromatolysis after lesions of the central processes is not due to the distance of the lesion from the cell body nor to time of survival since section of the peripheral processes equidistant from the ganglion and with identical survival time caused definite chromatolysis.

(102) *50. A new type of abdominal adhesion (opossum and monkey).* Carl G. Hartman, Department of Embryology, Carnegie Institution of Washington.

Among several hundred laparotomies performed on the opossum and on the monkey, slender adhesions between ovary and omentum have been observed in each of these species. Serial sections of the ovaries show that the adhesion is connected with a corpus albicans. A comparison of relatively fresh and older cases indicates that the adherent threads arise by cellular organization of strands issuing from ruptured follicles. Such strands may conceivably consist of blood fibrin or liquor folliculi, more probably the latter, which in fresh corpora lutea is often so viscous and tenacious that one may draw it out into a thread 12 inches long. The phenomenon has been observed in about 2% of females examined.

(117) *51. Quantitative changes in body growth and organ weights in young albino rats following total oophorectomy.* E. I. Hashimoto and Clay B. Freudenberger, Department of Anatomy, University of Utah Medical School.

Twenty-five Wistar albino rats were bilaterally ovariectomized at an average age of 26 days and necropsied at 70 days. Control and spayed body and organ measurements were made. Biometrical methods were applied in the analysis of the data; and these results, in turn, were compared with results from two groups of rats autopsied at 90 and 184 days, respectively.

In all the spayed animals the weights of the body, head, integument, humerus, thymus and stomach were increased; but only in the younger groups were the lengths of the body, tail, femur, humerus, and the weights of the femur, brain, hypophysis, heart, lungs, spleen, submaxillary glands, alimentary group, intestines, liver and kidneys increased.

Questionable alteration as a result of ovariectomy was found in the weights of the spinal cord at 70 days; and in tail length, and the weights of the heart and lungs at 184 days. The thyroid weight was questionably increased at 70 days, significantly heavier at 90 days, and unchanged at 184 days. The uterus was significantly smaller in all spayed animals. No changes occurred in the weights of the eyeballs. The suprarenal glands were decreased in weight at 90 and 184 days, but unchanged at 70 days.

(18) *52. Determination of the axis of the germ-disk and the first appearance of embryonic mesoblast in macaque embryos.* Chester H. Heuser, Department of Embryology, Carnegie Institution of Washington.

The formative cells of the 9-day blastocyst are clumped in a nearly spherical mass and one cannot recognize as yet any axial arrangement of its cells. In an embryo slightly older the formative cells are arranged as an oval, instead of a spherical mass, and the broader end of this oval mass probably represents the head end. In a series of fourteen embryos, from the ninth to the seventeenth day, without exception they all exhibit this axial elongation and the differentiation of the head end becomes progressively more distinct as the embryos become older. Therefore we must assume that its axis is determined before the embryo has acquired its flattened germ-disk character.

The primitive streak, appearing at first as an oval area, can be identified at about the thirteenth day. At this time mesoblast cells are present between gut entoderm and overlying ectoderm. Graphic reconstructions of the embryos show that the mesoblast cells are much more numerous in the caudal half of the germ-disk than elsewhere and from there spread laterally and forward as occurs in other mammals, notably in the pig. The primitive node appears later than the primitive streak. It is clearly present, however, on the seventeenth day.

(78) *53. Functional homeoplastic adrenal grafts in the white rat.* George M. Higgins and Dwight J. Ingle, University of Minnesota and Mayo Foundation.

Autografts of the adrenal gland are usually successful, but functional homeografts of the adult gland are rarely obtained. When donor and host are closely related, a higher percentage of successful homeografts is more likely to occur.

This report covers the results of transplanting adrenals of newborn rats to the groin of bilaterally adrenalectomized unrelated adult rats. Total adrenalectomy was performed on twenty-one adult male rats and at the same time, eight or ten adrenals of newborn rats were implanted to pockets in the groin, closely adjacent to the femoral vessels. Of this series nine rats survived the period of adrenal insufficiency and following an initial weight loss gained in weight and resembled unoperated controls. After 4 months, groins were explored and a cluster of six or seven glands was found at the site of implantation in each animal. To determine functional capacity, all such homeografts were carefully removed. All rats then showed characteristic symptoms of adrenal insufficiency and all were dead by the thirteenth day following exploration. The function of these homeografts seemed clearly established.

The biologic incompatibility normally encountered in homeografts of adult gland is not as prevalent in homeografts of the newborn gland. It is suggested that the organismal differentials, described by Loeb are sufficiently undeveloped in the newborn as to permit of adaptation of graft to host in homeotransplantation.

(123) *54. Ovaries secrete male hormone. II. Some factors involved in the male hormone output by grafted ovaries.* R. T. Hill, Department of Anatomy, Yale University School of Medicine.

Ovaries grafted into the ears of gonadectomized male mice secrete male hormone (Hill and Gardner, '36). Growth and activation of the castrate type of seminal vesicles and prostates is the criterion used. To get such a response certain definite conditions seem necessary.

Recordings by thermocouple of the temperature of several regions of mice have been made. The temperatures of the tip of the ear very closely approximate those of the tip of the scrotum; i.e., both average about 29 to 30°C. These temperatures range from 5 to 6°C. below that of the peritoneal cavity and of the subcutaneous regions of the abdomen.

With ovaries grafted in the warm regions of the peritoneal cavity or under the skin of the abdomen the atrophic seminal vesicles and prostates of the host were not stimulated. However, if the tip of the ear was used as a site for the graft (cool region) the output of male hormone was greatly accentuated. The presence of co-existing grafts of uterus and vagina did not affect the response obtained. The histology of the grafted ovaries did not explain their peculiar physiology.

Evidence so far obtained indicates that the low temperature to which an ovary grafted in the ear is subjected may be a critical factor in the elaboration of male hormone.

(9) *55. The inhibition of experimental menstruation by oestrin and progesterone.* Frederick L. Hisaw, R. O. Greep and H. L. Fevold, Harvard University.

The relative effects of estrin, progesterone and combinations of the two hormones on the inhibition of bleeding from an 'oestrin' endometrium was tested in thirty-eight experiments upon castrated monkeys. Each animal was prepared, immediately preceding the experiment, by giving 100 RU of oestrin daily for 19 to 21 days or by injecting pituitary extracts followed by castration. When oestrin was reduced to 25 RU daily bleeding began on sixth day; 50 RU, ninth to tenth day. Progesterone: ¼ RbU daily, bleeding began sixth day; ½ RbU, eighth to thirteenth day; ¾ RbU, fifteenth to twenty-fifth day; 1 RbU, 44 days without bleeding but menstruation began 4 to 7 days after the dosage was reduced to ½ RbU daily. Oestrin plus progesterone: 25 RU plus ¼ RbU daily, bleeding began eighth to fourteenth day; 25 RU plus ½ RbU, may be continued 18 to 22 days without bleeding; 50 RU plus ¼ RbU, bleeding may not occur within 28 days; and 50 RU plus ½ RbU, inhibited 20 to 22 days without bleeding. Dosages of oestrin or progesterone which will not inhibit menstruation when given alone will do so when the two hormones are injected simultaneously. Also, ¼ RbU of progesterone plus 25 to 50 RU of oestrin daily will not produce a fully developed premenstrual endometrium while ½ RbU plus the same dosage of oestrin will.

(91) *56. Microcinematographic and electrocardiographic records of the action of the embryonic chick heart.* Ebbe Hoff, T. C. Kramer and B. M. Patten, Department of Physiology, Yale University and Department of Anatomy, University of Michigan.

The work to be reported represents an attempt to secure a series of electrocardiograms during the period of the transformation of the simple tubular embryonic heart into its four-chambered condition, and to compare such electrical records with microcinematographic records showing the character and manner of spreading of the heart beat. The microcinematographic technique employed was that described by Patten and Kramer ('32, '33). In making the electrical records (Hoff and Kramer), the potential changes were led off by microelectrodes, passed through a three-stage amplifier, and recorded with a Hindle electrocardiograph. The first arrhythmic fibrillar contractions have yielded fair photographic records but as yet no satisfactory electrical tracings. Workable records of both types have been obtained from embryos as young as 31 to 32 hours, i.e., during the period when the heart is first settling into rhythmic pulsation, well before the actual circulation of blood has commenced. The electrical records at this stage show simple diphasic waves. A suggestion of polyphasic configuration begins to appear coincidentally with the marked bending of the cardiac tube, at about the time the atrium becomes active in contraction and the circulation of blood commences. Shortly thereafter, there appear deflections which seem to be comparable with the P-wave and the QRS complex of adult electrocardiograms. From the third day onward, the electrocardiograms progressively approach the configuration of adult tracings.

(114) *57. A comparison of the rates of absorption and callus stimulating properties of cow-horn, ivory, beef-bone and autogenous pegs inserted into bone cortex.* Charles W. Hughes, Department of Anatomy, Loyola University School of Medicine. (Introduced by R. M. Strong.)

Cow-horn, ivory, beef-bone and autogenous pegs 1.5 mm. in diameter and approximately 3 mm. long were inserted by aseptic technique into twelve dog femora, eight dog radii and eight rabbit femora. The pegs were placed in alternating positions into holes drilled through the bone cortex at intervals of 10 to 15 mm. The pegs were inserted so that the middle millimeter of each was surrounded by cortex. One millimeter of each peg extended into the medullary cavity and the remaining 1 mm. projected above the periosteum. Roentgenograms were taken frequently. The animals were allowed to live periods of time varying from 1 week to 1 year. Photographs, roentgenograms and histological sections were made when the bones were obtained after death of the animals.

The autogenous, beef-bone, ivory and cow-horn pegs showed signs of beginning absorption in the order mentioned. The extracortical portion of each peg was the first absorbed; the intramedullary portion was absorbed next and the intracortical portion persisted longest.

The roentgenograms revealed 25% less callus in the vicinity of the autogenous peg than around the ivory, beef-bone and cow-horn pegs which were surrounded by equal amounts of callus. (See demonstration no. D28.)

(124) *58. The inhibition of immature amphibian gonads by gonads of the same sex type.* R. R. Humphrey, Department of Anatomy, University of Buffalo.

The volume of the testis in Amblystoma punctatum (maculatum) at 2.5, 4 and 6 months has been determined for normal and hypophysectomized controls, normal and hypophysectomized males carrying large gonad grafts (from A. tigrinum donors), and normal and hypophysectomized unilateral castrates.

The testis volume in hypophysectomized controls does not appear significantly lower than that in the normal controls in any of the three age groups studied. Effective stimulation by gonadotropic pituitary hormone may therefore be regarded as lacking during this period.

The average testis volume in both normal and hypophysectomized males carrying testis grafts is significantly reduced in all three age groups. The testis volume in both normal and hypophysectomized males of 4 and 6 months is significantly increased in comparison with the corresponding controls when the graft is an ovary instead of a testis. The volume of the testis in unilateral castrates is also significantly larger than that of controls, both in normal and hypophysectomized males. The smaller size of the testis in males with testis grafts and in controls as compared with males carrying graft ovaries or males unilaterally castrated, indicates an inhibition of one testis through some influence from another. It is suggested that this inhibition may be mediated through the agency of the same inductive substance (medullarin-; Witschi) by which the testis inhibits an ovary during early development.

(103) *59. Differential counts of the islet cells of the dog pancreas.* Thomas E. Hunt, Department of Anatomy, University of Alabama.

Differential counts of the alpha, beta (insulin) and delta (Bloom) cells of four dog pancreases have been made, the respective average percentages being approximately 20, 75 and 5. The tissues from which the counts were made were taken from various regions of the tail, body, head and uncinate process. After fixing the tissue in Zenker-formol, 4 μ sections were obtained and stained with the Mallory-azan technique. The nuclei of all the islet cells from one section of each block were counted. The percentages vary slightly in the different regions and in the different animals and greatly in the individual islets. To date approximately 1500 islets with an average of thirty cells per islet have been counted.

(54) *60. The effect of the size of the container on the growth of larvae of Amblystoma tigrinum.* Cranford Hutchinson, Morris Biological Farm of The Wistar Institute.

Since larvae of Amblystoma tigrinum maximally fed in the laboratory grow less rapidly than larvae growing in their natural habitat, it seemed that exercise might be a growth promoting factor. To test this, three groups of similar A. tigrinum larvae were raised in different sized containers. Group I, thirty individuals in separate finger bowls with 250 cc. of water and 95 sq.c. of open surface in each bowl; group II, sixty individuals together in one large aquarium with 250 cc. of water and 50 sq.c. of surface per animal; group III, thirty individuals together in a wooden tank with 2558 cc. of water and 284 sq.c. of open surface per animal. All groups were maximally fed on similar diets. Group II, which had no greater volume or surface area of water per animal but which had room in which to exercise, grew significantly faster than group I which was raised in individual finger bowls. Group III, which was raised in a relatively greater volume of water than group II, grew significantly faster than group II during the latter half of the experiment. Both groups II and III grew faster than the recorded growth for this species in nature which indicates that exercise or some result of mass culturing affects the growth rate.

(31) *61. The effect of nicotine upon coeliac ganglion cells.* E. H. Ingersoll, Department of Anatomy, Medical College of Virginia, Richmond. (Introduced by H. L. Osterud.)

It has been shown previously that the coeliac ganglion cells of rabbits which have been injected subcutaneously with nicotine sulphate for short periods of time were within the limits of normal cells. In animals, however, that have received daily or twice daily injections of nicotine for periods of 2 weeks to 170 days the ganglion cells vary somewhat from the normal picture. There is a decline in the types of cells having a well distributed, definitely granular Nissl substance and an increase in the types of cells characterized by a peripheral zone of Nissl substance. The number of senile and exhausted cells is not appreciably increased but in a majority of the ganglion cells of chronic nicotized animals the Nissl granules are not as large and distinct and do not stain as well as in the controls or in those receiving injections for short periods of time. In addition to these changes there is an increase in the number of cells with eccentrically placed nuclei, evidence of neuronphagia and a slight proliferation of neuroglia.

This report is a summary of work on forty-three rabbits with a total classified cell count of over 41,000 ganglion cells.

(55) *62. Recovery of rats upon refeeding after prolonged suppression of growth by underfeeding.* C. M. Jackson, Department of Anatomy, University of Minnesota.

Male albino rats were underfed so as to hold them at nearly constant weight of about 54 gm. from weaning (age 3 weeks) to age of 18 weeks. Mortality was high (42%). The thirty-eight survivors were than fed ad libitum on the same stock diet. They grew at first rapidly, then more slowly. Their final weight at age 14 months averaged 400 gm., considerably below that of forty-four normal litter-mate controls (average 477 gm.) which were fully fed on the stock diet throughout. There were marked individual differences, however, and a few test rats ultimately equalled the controls.

At final autopsy, the test rats, although lower in body weight, had organ weights nearly equal to those of the normal controls for brain, hypophysis, submaxillary gland, heart, lungs, stomach, intestines, kidneys, testes and epididymides. The other organ weights (skeleton, muscle, eyeballs, thyroid, thymus, suprarenals, liver, spleen), also body and tail lengths, averaged below the controls, although about normal for corresponding body weight.

Various investigators have disagreed concerning the ultimate effects of early inanition upon later capacity for growth. The present results support the view that severe and prolonged inanition during early life may prevent full completion of growth later and thus cause permanent dwarfing. These results through simple underfeeding are more pronounced than those previously reported where growth was suppressed by dietary deficiency of protein.

(51) *63. Anatomy of the inguinal region.* T. T. Job and Frank J. Nowak, Department of Anatomy, Loyola University.

An attempt has been made to correlate the various structural conditions found in the lower quadrants of the abdominal wall with the degree of muscular development in the wall, by dissections (binocular), measurements and trans-illumination.

Material: 177 cadavers (95 white ♂, 56 colored ♂, 7 white ♀, 19 colored ♀). Data so far indicate: external oblique m., shows no considerable variation with regard to side, sex or race, with almost one-third of specimens showing better than average development (textbook descriptions are taken as average); internal oblique m., much better developed in females, least developed in white males, best developed in colored females, with about one-half of white males, one-third of colored males, three-fourths of females showing better than average development; transversus m., also better developed in females, and weakest in white males. There is no correlation between size of the external ring and degree of muscular development, but large rings are more frequently associated with peritoneal diverticulae in the internal ring. Diverticulae are more frequent on the right side and very much more common in males, particularly white males (51% right, 31% left).

In forty-two white and twenty-one colored males, fibers from the ilio-pectineal line extending into Hesselbach's triangle were present in one-half of the specimens. Interfoveolaris and pubotransversalis muscles were found in 13%. Hesselbach's and Henle's ligaments were found in 56%. Special developments of the internal oblique related to the inguinal canal are found in 43%.

(35) *64. On the histogenesis of the sympathetic trunks in the chick embryo.* David S. Jones, Department of Anatomy, University of Minnesota. (Introduced by E. A. Boyden.)

By removal of neural crest cells and dorsal portions or whole segments of the neural tube in chick embryos, an attempt was made to discover the source of cells which form the sympathetic trunks. Eggs were incubated for more than 40 hours so that operations could be performed in the thoracolumbar region. The operations consisted of cutting the ectoderm on either side of the neural tube and removing the dorsal portion of the tube, or all of the tube, with overlying ectoderm, from as many segments as possible. The eggs were reincubated 3 to 8 days and prepared for study by Cajal's technique. In the operated region the spinal ganglia and longitudinal fibers in the dorsal funiculus were always absent, yet the ventral half of the neural tube, the ventral roots and the sympathetic trunks were normal. The spinal ganglia were absent in the operated field in each of twenty embryos studied. In the most favorable case as many as eight spinal ganglia were missing; notwithstanding, the sympathetic trunks were normal. In portions of the embryo where the neural tube was absent for two or three segments, the sympathetic ganglia were connected by ventral roots to the tube at another level. Cresyl violet preparations of normal 3-day embryos show the ventral roots as chains of cells continuous with the neural tube. (See demonstration no. D29.)

(149) *65. The hypothalamic autonomic centers.* C. U. Ariëns Kappers, Central Institute of Brain Research and University of Amsterdam.

Doctor Kappers will discuss a series of photomicrographs concerning the autonomic hypothalamic centers, their relation to blood vessels and to the ventricle, including some of their afferent and efferent connections in fishes, reptiles, lower and higher mammals, and man.

(12) *66. The development of the megakaryocyte in the embryonic rabbit.* Daniel M. Kingsley, Department of Anatomy, Louisiana State University Medical Center.

The development of the megakaryocyte was studied in the livers of rabbit embryos. The technic for the preparation of this material was that already used for a previous study of megakaryocytes in the embryonic pig. In the livers examined, megakaryocytes were found to be far more numerous than generally believed. By selecting early stages of development, it was possible to observe hemopoiesis limited to the erythropoietic and megakaryopoietic series of cells. Megakaryocytes were then seen to develop from typical hemocytoblasts in which the first change toward a megakaryocyte was the presence of a few typical granules in a perinuclear cap of cytoplasm. Both nucleus and cytoplasm then hypertrophy, and the number of granules increases. Such stages of development are common. Subsequent growth causes complex lobulation of the nucleus and the cytoplasm extends in thin layers between surrounding marrow cells. Mitoses are not common but are present. Pseudopod formation occurs early. No agranular 'megakaryoblast' stages were observed. The principles of megakaryopoiesis in the rabbit therefore conform to those already described for the pig.

(11) *67. The red blood cells of mammalian embryos.* Arthur Kirschbaum, Department of Anatomy, University of Minnesota. (Introduced by Hal Downey.)

The dry imprint method was used in the study of embryonic blood forming organs. Observations were correlated with the interpretations of hematologists using sectioned material only (strict unitarians).

The first immature blood cells of the rat yolk sac are not lymphocytes, but basophilic megaloblasts quite similar to the pathologic megaloblasts of pernicious anemia marrow. The red cell series of embryonic liver, spleen, fetal and adult bone marrow is cytologically different from the yolk sac megaloblastic line. The nuclear pattern of immature embryonic megaloblasts, nuclear retention in many until removed from the blood, and the large size of orthochromatic elements, either non-nucleated or with small pyknotic nuclei, differentiate them from the normoblastic series (embryonic liver, etc.). There are separate young (basophilic, polychromatic) and old (fully hemoglobiniferous) elements of both the megaloblastic and normoblastic series, which represent two distinct cell lines. Megaloblastic red cells are formed normally only in early embryos; they are not encountered subsequently in normal hemopoietic organs. Where the gestation period is short (rat), such cells circulate up to birth. They disappear from the human fetal circulation, however, by 4 months. The 'megaloblast' of Maximow, Jolly, Bloom and Jordan is a small basophilic normoblast and not the megaloblast originally described by Ehrlich for the early embryo and pernicious anemia. Sabin's 'megaloblast' (as well as Osgood's) is any basophilic red cell, whether found in the embryo, normal or pathologic adult. (See demonstration no. D32.)

(80) *68. Intensity of activity of adrenal medulla in poikilothermy and hyperemotional states following diencephalic lesions.* Wendell J. S. Krieg, Department of Anatomy, College of Medicine, New York University.

In seventy-nine male albino rats a portion of the diencephalon was injured by the injection of a droplet of mercury into the third ventricle, or partially destroyed by a minute cautery. The animals here considered developed either 1) poikilothermy, or 2) a hyperemotional state characterized by the inordinate expression of rage and flight on being approached even quietly.

The adrenals of these, together with the adrenals of operated negatives and unoperated controls were fixed by Regaud's method, sectioned, and either 1) mounted unstained, or 2) stained with anilin blue, or 3) bleached and stained for mitochondria.

Adrenal medullae of poikilotherms uniformly differed widely from those of any other class. Glands of rage-flight animals varied as widely as did controls and negatives and in no case did their picture resemble that of adrenals of poikilotherms.

Thus the activity of the adrenal medulla of poikilotherms is profoundly altered, while in rage-flight animals, whether or not they are excited before killing, there is no uniformly heightened state of activity, as judged by anatomic criteria.

(60) *69. The ontogenetic development and phylogenetic significance of the cortex pallii in the chick.* Hartwig Kuhlenbeck, Department of Anatomy, Woman's Medical College of Pennsylvania.

During the fourth day of incubation the longitudinal zonal pattern of the telencephalon becomes distinct in the chick. The zones D1, D2, D3 of the pallium and B1, B2, B3 of the basis telencephali show an arrangement directly comparable to the pattern of anamnia (amphibians).

Passing through a developmental stage exhibiting features common to reptiles and birds the outlines of the definite avian telencephalic pattern are vaguely but unmistakably taking shape between the eighth and tenth day. At the twelfth day the fundamental arrangement can be regarded as completed. Until hatching a gradual differentiation of architectural details goes on.

D3 constitutes the hippocampal formation and corresponds to the medial cortical plate of reptiles, the dorsomedial subdivision of D2 becomes cortical and represents the parahippocampal cortex comparable to the medial part of the dorsal cortical plate of reptiles. The lateral subdivision of D2 forms two noncortical nuclei corresponding to the primordium neopallii and to the anterodorsal part of the lateral cortical plate of reptiles. D2 in fusing with D1 differentiates a corticoid layer becoming more cortical caudally and extending upon the surface of D1, comparable to the ventral and caudal parts of the lateral cortical lamina of reptiles.

(29) *70. Structural organization of the celiac ganglia.* Albert Kuntz, Department of Microanatomy, St. Louis University School of Medicine.

The distribution of nerve fibers of various morphological types in relation to the ganglion cells is considered on the basis of observations on preparations of ganglia obtained from human cadavera and unoperated cats and ganglia obtained from cats following degeneration of the splanchnic nerves alone and following degeneration of both the splanchnic and vagus nerve fibers which join the celiac plexus. Some synaptic fiber terminations in the celiac ganglia remain intact following section and degeneration of only the splanchnic nerves.

(43) *71. Estimation of the effect of prolactin on the growth of the pigeon's crop gland by the colchicine technique.* C. P. Leblond and Edgar Allen, Department of Anatomy, Yale University School of Medicine.

Prolactin has a double effect on the crop gland of the pigeon, namely stimulation of the cell multiplication in the epithelium and intracellular deposition of fat granules. The cellular multiplication may be accentuated by the use of colchicine. Following injections of this alkaloid, mitoses are held and accumulated at the metaphase stage, their number being maximal after 10 hours.

Sections of the growing crop gland of animals treated with prolactin alone show less than 1% of the cells in mitosis. Resting crop glands of control animals given colchicine alone, contain ½ to 2% of the cells arrested in mitosis. In a pigeon treated with prolactin and colchicine, from 10 to 53% of the cells are dividing. Such a number of mitoses would be (Ludford, Brues) a true picture of the number of cells which would have completed division during the time of action of colchicine (10 hours).

Nine hours after injection of prolactin a definite growth effect may be detected by the colchicine method. The maximal growth seems to occur after 36 hours. In animals treated by prolactin for this time, the ratio of dividing nuclei to the total number of nuclei of the epithelium, has been used as an index of the growth action of prolactin (mitotic index).

Colchicine has also been successfully used to estimate the crop gland growth induced by local injections of prolactin or implants of pituitary tissue under the skin of the crop gland.

(108) *72. The continuity of the Golgi apparatus in animal cells.* T. J. Macdougald, Department of Zoölogy, Trinity College, Dublin and Department of Biology, Washington Square College, New York University. (Introduced by Robert Chambers.)

The mechanism by which the Golgi apparatus is distributed to the daughter cells during mitosis, especially in tissue cultures, is as follows: Before the breakdown of the nuclear membrane, the Golgi apparatus, at first a single reticulum, becomes divided into two approximately equal portions which migrate until they come to lie at opposite poles of the nucleus. After breakdown of the nuclear membrane and formation of the metaphase spindle, the Golgi material, which now consists of granules, lies close to the centrosome at each pole of the spindle. As the chromosomes move to the poles at anaphase and the daughter cells are formed, each cell receives its complement of Golgi material. With the reconstruction of the nucleus at telophase, a bipolar Golgi apparatus is at first formed, which later reverts to the single network type of the resting cell. In cells vitally stained with neutral red, the stained granules occupy a different locality from that of the Golgi apparatus and remain distinct from it during all stages of mitosis.

(3) *73. The one-cell-type conception of the definitive mammalian pulmonic alveolar wall structure.* Charles C. Macklin, University of Western Ontario.

The abstract of my (unread) Milan Congress paper begins thus: "The wall of the pulmonic alveolus in the domestic cat, rabbit and man is composed of syncytially joined flattened septal cells, reinforced by elastic and reticular fibrils, and tunneled by capillaries, whose walls are condensations of the protoplasm." Scattered cells, seeming to correspond to the 'epicytes' of Clara ('36) are found in the capillary meshes. They resemble, in nuclear and cytoplasmic structure and staining, the continuous epithelium of the respiratory bronchioles. Though never forming a continuous layer on the wall of the definitive alveolus, their apposed cell edges could be demarcated by silver impregnation where they occupied the marginal zone leading out of a respiratory bronchiole (homo) and here the 'pavement' became broken up. Except for these relatively few, isolated, apparently entodermal elements, which sometimes show mitotic figures, the optical appearances, after a variety of fixations and stainings, suggest that the cells of the fully developed mammalian alveolar wall are of a single (mesodermal) type. These septal cells, and their specialized exoplasm, or 'ground substance,' form the essential wall. The air spaces may be termed a 'functional interstitial emphysema'; though it is possible that the air contact surface has undergone a structural specialization. (See demonstration no. D34.)

(13) *74. Abnormal blood production in hydrops fetalis.* Madge Thurlow Macklin, University of Western Ontario. (Introduced by Charles C. Macklin.)

The erythroblastosis found in congenital anemia, fatal icterus of the newborn and hydrops fetalis has been attributed to the inability of the child to adjust its blood production to the high oxygen content of its postnatal environment. Examination of the blood in a 6 months' hydropic fetus indicates that in hydrops, at least, the difficulty is due not merely to lack of physiological adjustment but to a profound developmental anomaly which exists long before birth, and affects the structure of the chorionic villus as well as blood production in the fetus. It is suggested that the high number of pregnancies terminating early in the mothers who give birth to hydropic fetuses are examples of the same profound developmental disturbance. A genetic relationship between the three above-mentioned conditions which exhibit erythroblastosis is postulated. (See demonstration no. D35.)

(66) *75. Descending connections from the hypothalamus.* H. W. Magoun, Institute of Neurology, Northwestern University Medical School.

The location in the brain stem of the cat of descending connections mediating influences from the hypothalamus has been investigated by observing the effects of hypothalamic stimulation after partial sections at the midbrain and pontile levels. Representative effects have been studied: the sympathetic by vasomotor responses, parasympathetic by contractions of the urinary bladder, and somatic by respiratory reactions.

In the midbrain descending connections which mediate impulses induced by hypothalamic stimulation occupy a wide area in both the central and tegmental regions.

At the pontile level the results fail to support any conception of an exclusive or predominant disposition of descending connections in the midline region. Some connections are present in the medial portion of the pontile brain stem, but sections must be extended laterally practically to the lateral margins of the tegmental formation to seriously impair the responses to hypothalamic stimulation or to abolish them. If there is any concentration of descending connections at this level these experiments would point toward the pontile tegmentum as the area concerned.

Stimulation after hemisections reveals that even as far caudad as the first cervical segment of the spinal cord, the connections which carry descending impulses from the hypothalamus are chiefly uncrossed, but there is a smaller crossed component.

(7) *76. The Falkiner ovum.* C. P. Martin, Department of Anatomy, McGill University, Montreal. (Introduced by H. E. Rawlinson.)

This ovum formed the subject of a previous publication which dealt almost exclusively with the formation of the placenta and the vascular arrangements in the maternal tissue. No description of the actual embryo itself has yet been published. Through the kindness of Doctor Falkiner the specimen has been given to the author for the purpose of preparing a detailed description of the embryo. The age of the specimen is estimated at about 15 days and it manifests some peculiarities, notably a tendency to subdivision of the yolk sac and in the structure of the allantois which originates low down in the caudal wall of the yolk sac.

(23) *77. The otic region of a 29-mm. (CR) human embryo.* John Martin, Department of Surgery, Northwestern University Medical School. (Introduced by Barry J. Anson.)

The capsule at 29 mm. has attained complete chondrification except for a small area in front of the anterior crus of the lateral semicircular canal. The ossicles are in a rudimentary state, the stapes being a simple ring of cartilage deeply set into the irregularly oval fenestra. The boundaries of the fenestra cochleae are still incomplete; the so-called 'supra-cochlear' cartilages are not identifiable; otherwise the anatomy is that described earlier by Professor Macklin for the embryo of similar length.

The membranous labyrinth has attained a definitely adult form, in contrast to the primitive state of the capsule and ossicles. The endolymphatic sac lies in a bed of richly vascular connective tissue which surrounds the transverse sinus; it bears a filliform elongation within which is a minute lumen. Vacuolization, which will result in the formation of the periotic spaces, is limited to the vestibule immediately behind the base of the stapes, an observation corroborative of Professor Streeter's studies.

The internal auditory meatus contains three passages; one for the facial nerve and superior division of the vestibular nerve; a second for the nerve of the canalis singularis and inferior division of the vestibular nerve; a third for the cochlear nerve.

Our series will ultimately include all important stages in development of the capsule, up to those already described by Professor Bast.

(17) *78. The significance of the embryonic area in the opossum.* Edward McCrady, Jr., The Wistar Institute of Anatomy and Biology.

The unilaminar blastocyst contains no inner cell mass and shows no differentiation into animal and vegetal poles or embryonic and trophoblastic regions. The first step in visible differentiation is the enlargement of certain cells and their migration into the blastocoel. These represent the endoderm and some axial mesoderm (viz. the prechordal plate material which is later found in the endodermal layer proliferating mesoderm into the head region). At the point where the 'invaginated' material makes contact with the external layer the latter (probably as the result of induction) forms a conspicuous plate of denser cells which by former authors has been called the 'embryonic area.' The primitive streak appears in the posterior tip of this area, which tip becomes the sinus rhomboidalis. When the embryonic body wall forms, it is decidedly lateral to the so-called 'embryonic area,' and the latter turns out to be only a medullary plate. At no stage in the opossum embryology is there any histological distinction between embryonic and extraembryonic cells in any of the germ layers. The entire ovum is homologous with that of any of the lower vertebrates. The appearance of the medullary plate by induction immediately after 'gastrulation' is exactly what one would expect on the basis of the facts derived from descriptive and experimental embryology of lower vertebrates, and makes the mammalian ovum fit naturally into the phylogenetic series.

(59) *79. Description of the brain of a human cyclopian monster.* Fred A. Mettler and Cecilia C. Mettler, Department of Anatomy, University of Georgia School of Medicine.

The subject of this study was a human full-term foetus with a single median eye and no external nasal apparatus either of the usual or snout form. The eye was symmetrical and set in a palpebral fissure apparently constituted of the lateral halves of two fused sets of palpebrae. The exact age and history of the foetus is not known having been acquired in 1832 and assumed to have been bought in Paris by L. A. Dugas who was commissioned to collect a museum for the medical college at that time.

The specimen apparently had been preserved in 'pyroligneous acid' and is in a good state of internal as well as external preservation. Only the cranial cavity was opened for this study it being desired to preserve the external appearance as closely as possible.

The pallium was found to consist of an undivided horseshoe-shaped structure encircling the thalamic mass above which a cystic vesicle represented the hydrocephalic condition so often found associated with cyclopia. No definite rhinencephalon existed. The cranial nerves after the optic were not peculiar in their mode of origin.

Microscopically the cellular structures were found to be poorly preserved but a fair degree of success with the Weigert method was obtained. The results are in accord with the findings of D. Davidson Black.

(85) *80. Some effects of male and female sex hormones on the reproductive system of the hypophysectomized male rat.* Warren O. Nelson, Wayne University, School of Medicine.

It has been shown that the administration of extracts of male hormone will, for at least 60 days, almost completely protect against the degenerative changes which occur in the seminiferous tubules after hypophysectomy. To obtain this effect injections must be begun within 10 days after hypophysectomy. The testes remain in the scortum, are of almost normal size, and show normal spermatogenesis. Sperm motility is excellent, the sex-accessories are normal, and fecundation of normal females has been observed. The interstitial cells are not stimulated by this treatment.

The testes of males allowed to go untreated for more than 10 days after hypophysectomy are not protected (maximum dosage has been 25 B.U. daily). The testes of animals allowed to go untreated for 21 days and subsequently injected with gonadotropic hormone for 20 days are returned to a normal condition. The substitution of male-hormone extracts for gonadotropic hormone in such animals results in testicular maintenance.

Four synthetic androgenic substances, viz., testosterone, androsterone, dehydroandrosterone, and androstone-dione give responses similar to male-hormone extracts. This evidence, as well as specific tests of the male-hormone extracts, show that testicular maintenance is not due to the presence of gonadotropic hormone.

Progestin has been as effective as male hormone in maintaining the testes for as long as 25 days after hypophysectomy. Although experiments with crystalline progesterone have not as yet been completed, and as it is possible that the progestin extracts contained certain substances which have male-hormone activity, the condition of the sex accessories would indicate that male-hormone activity per se is not the factor responsible for maintenance of the testes in these animals.

Several estrogenic hormones have been shown to have no capacity to maintain the testes in hypophysectomized animals.

Consideration is given to the relation of such factors as gonadotropic hormones, male hormones, scrotal maintenance, etc., in spermatogenesis.

(52) *81. The pre-splenic fold in the adult.* H. D. O'Brien, Department of Anatomy, McGill University, Montreal. (Introduced by H. E. Rawlinson.)

This fold was first described by Reid in 1912, since when no reference appears to have been made to it. He described it in the human embryo, and noted its presence in the normal adult, calling it the pre-splenic fold, and its sinistral prolongation the sub-splenic fold.

The frequent occurrence of this fold in the normal adult, and the absence of any description of it in the standard textbooks led to an analysis of its incidence in thirty dissecting room subjects.

The fold was found in practically every case, and varied from a ridge ½ inch high to a fold 3 inches high, sometimes containing a prolongation of the lesser sac. Typically it is attached to the anterior lamella of the gastro-splenic ligament from the region of the phrenico-colic ligament upward to the region of the fundus. Inferiorly it divides into two smaller folds, of which the larger, sub-splenic, runs to the left under the spleen, while the smaller fades out on the anterior surface of the great omentum. In a few cases it contains a prolongation of the lesser sac bounded below and to the right by a well-marked fold containing the left gastro-epiploic artery. It is supplied by one or more branches of the splenic or gastro-epiploic artery.

Its frequent occurrence appears to warrant textbook description.

(87) *82. Histological variability of the human testis.* Harald Okkels, University of Copenhagen and Department of Anatomy, College of Physicians and Surgeons, Columbia University. (Introduced by Earl T. Engle.)

The present report deals with the findings in testes from thirty-nine individuals with psycho-sexual aberrations, castrated by the authority of the Danish sterilization act of June 1, 1929. The medical aspects of the law, and this study at the Institute of Forensic Medicine, University of Copenhagen, is under the direction of Dr. Knud Sand. In thirty-three cases of sudden death (accident or suicide), where autopsy did not reveal organic lesions of any importance, the testes were secured to afford an approximation to a control group. The cases are mostly in the 30- to 40-year age group. The weight of the testes is highly variable. Nearly two-thirds of all the testes showed varying degrees of regressive changes of the seminiferous epithelium; cellular degeneration, atrophy and cessation of spermatogenesis in the affected tubules. About one-third of all the testes showed fibrous, hyaline or edematous alterations of the connective tissue; lymphocytic infiltrations were exceptional; slight vascular alterations were found in a few cases. In several cases, the interstitial cells appeared to be more abundant than is usually considered normal. Our observations suggest that the histological aspect of the 'normal state' of the human testis is more highly variable than previously thought. All the individuals in question might have passed an ordinary medical examination as healthy subjects, with no indication of structural pathology.

(15) ***83. Behavior of the isolated embryonic chick heart in ouabain.*** George H. Paff and J. Raymond Johnson, Departments of Anatomy and of Physiology, Long Island College of Medicine. (Introduced by E. D. Congdon.)

Isolated 48-hour embryonic chick hearts subjected to solutions of ouabain behave as follows: 1) The heart rate accelerates preceding the appearance of a partial block of the ventricle. 2) The degree of the block progresses until the ventricle stops completely. Accompanying these changes in the ventricle the diastolic diameter gradually decreases. 3) The beat of sino-atrium, unlike that of the ventricle, shows no marked irregularities but becomes gradually weaker until it ceases. It invariably persists for a longer time than does the ventricular beat.

When the ventricle is separated from the sino-atrium and is subjected to the same treatment it shows an abrupt change in rhythm. The time required for the appearance of this change corresponds closely to that required for the appearance of block in the entire heart.

That these reactions of the heart in ouabain are to some extent reversible is shown by the fact that the ventricle, after complete stoppage, can be revived and a normal rhythm reestablished by washing with Tyrode's solution.

(46) *84. Differences in the reactions of normal and transplanted ovaries in guinea pigs to pregnancy urine hormone (follutein).* George N. Papanicolaou and Emil A. Falk, Departments of Anatomy and Medicine, Cornell University Medical College, New York City.

Autotransplanted ovaries in guinea pigs react differently to comparable dosages of the gonadotropic hormone of pregnancy urine (follutein) than do undisturbed ovaries.

The transplanted ovaries show greater tendency to the formation of cysts. This tendency is more pronounced in adult than in young animals. It is not uncommon to find transplanted ovaries which have become entirely cystic after prolonged treatment. In the normally situated ovaries the tendency to cyst formation, although increased after treatment, is much less pronounced. On the other hand, the stimulating effect upon the interstitial and thecal cells is more marked in the normally situated ovaries than in the transplanted ovaries, with corresponding dosages.

The cysts are derived from the rete ovarii tubules and their growth is closely associated with the growth and infiltration of connective tissue by which they are surrounded. The implanted ovaries being entirely embedded in connective tissue offer an easier access to its infiltration into the ovarian stroma and, thereby, a more favorable environment for the development and expansion of cysts.

The cystic proliferation induced by treatment is accompanied by an increased secretory activity of the epithelium lining the cysts. This results in a stimulation of the accessory female sex organs comparable to that produced by increased oestrin secretion.

(110) *85. Studies on the production of antibodies in vitro.* Raymond C. Parker, The Rockefeller Institute, New York.

Fragments of adult rabbit spleen removed from animals injected 2 and 3 days previously with washed guinea pig red cells produce easily demonstrable agglutinins against guinea pig red cells after 4 days incubation in a fluid medium. The medium consists of 3 parts normal rabbit serum, 1 part isotonic sodium bicarbonate (1.4%), 2 parts Tyrode's solution containing four times the usual amount of glucose, and 0.005% phenol red to serve as an indicator. For each culture, approximately 100 mg. of tissue (seventy-five fragments) are suspended in 2 cc. of medium contained in H-8 Carrel flasks. These flasks have a total capacity of 70 cc. In addition to the fluid medium, each culture is supplied daily with a gaseous atmosphere consisting of 80% O_2, 8% CO_2 and 12% N. Ordinarily, control cultures kept at 4°C. yield negative tests.

Under the same conditions of cultivation, adult rabbit spleens taken from animals injected 1 to 24 hours previously with washed guinea pig red cells failed to produce demonstrable antibodies. Negative results were also obtained when the spleen of such animals were cultivated in the sera of rabbits injected 3 days previously with the same antigen in the same amount. Some of the experiments have also been performed using bacterial antigens.

The experiments demonstrate that antibodies are readily produced in vitro after the antigen has remained for a certain period in the animal (See demonstration no. D43.)

(67) *86. Trans-neural atrophy of the nucleus ovoideus following eye removal in cats.* John R. Pate, Department of Anatomy, George Washington University Medical School. (Introduced by G. B. Jenkins.)

The nucleus ovoideus, a small group of closely packed cells lying just dorsal to the optic chiasma and lateral to the tip of the third ventricle, undergoes mild atrophy following eye removal in cats. There is a diminution in the size of the individual cells and the entire nuclear group presents an appearance of atrophy.

The right eye was removed from cats 1 and 2; the left eye from cats 3 and 4; and both eyes from cats 5 and 6. Cats 1, 2, 3, 4, 5 and 6 were allowed to survive for 72, 83, 71, 69, 70 and 69 days, respectively. All brains were fixed in 10% formalin, embedded in nitrocellulose, serially sectioned, and stained alternately with Weil and cresyl violet stains.

In cresyl violet stained sections, the contralateral nucleus ovoideus always presented an appearance suggesting transneural atrophy following unilateral eye removal. Following bilateral enucleation both the right and left nucleus ovoideus appear atrophic.

From this evidence it seems likely that some fibers of retinal origin effect synapses with the cell bodies of the contralateral nucleus ovoideus after crossing in the optic chiasma. In normal Weil stained preparations it is evident that a number of chiasmal fibers are directed dorsally in the immediate region of the nucleus ovoideus. What optic function this nucleus may subserve is not at present known.

(33) *87. Observations on the spinal accessory nerve in human embryos.* Anthony A. Pearson, Department of Anatomy, The University of Chicago. (Introduced by C. J. Herrick.)

The course of the spinal portion of the accessory nerve was studied in silver preparations of human embryos. The relation of this nerve to the ganglion and the dorsal root of the first cervical nerve was found to be variable. When the first cervical ganglion is present, the main trunk of the accessory may pass through it. In certain of the preparations ganglion cells can be seen along the intracranial portion of spinal XI, thus supporting the view that, at least in some embryos, this is a mixed nerve, containing a sensory as well as a motor component. In one embryo at the level of the first cervical segment it was possible to trace rootlets of the spinal accessory not only into their motor cell columns, but also to trace rootlets dorsad into the caudal end of the fasciculus solitarius where they descend (as was observed in the cat embryo by Windle).

(6) *88. A human embryo of two somites, in situ.* W. H. Piersol, Department of Anatomy, University of Toronto.

The embryo measures 2.03 × 0.72 mm. and has been sectioned sagittally. Contact between embryonic and maternal tissue is preserved over a considerable area although elsewhere there is separation due to shrinkage. Numerous glands form a broad stratum spongiosum. Flattened in the stratum compactum, their middle parts are much dilated; toward the muscularis they are again flattened. Frequently they contain degenerated detritus and occasionally blood. A few glandular remains occur in the peripheral parts of the decidua capsularis. Chorionic villi are longest and most numerous toward the decidua marginalis. Their tips may reach the maternal tissues or a spongy mass of trophoblast may intervene. Cytotrophoblast predominates, frequently bordered and penetrated by plasmodiotrophoblast. On the chorionic wall the trophoblast may show only one layer of nuclei but two layers are the rule. On the villi there are two layers of nuclei, the inner ones frequently belonging to distinct cells. The pericardial cavity is present but contains no heart, nor are their blood vessels in any part of the embryo, the chorion, or its villi. The neurenteric canal is closed but its remains are evident. An endodermal allantois projects into the connecting stalk. Small blood islands are present in the wall of the yolk sac and larger ones in the connecting stalk; the latter are forming endothelium.

(58) *89. Notes on an African elephant.* D. P. Quiring, Department of Biology, Western Reserve University, and Department of Anatomy, Cleveland Clinic Foundation. (Introduced by George Crile.)

The partial dissection of an enormous male elephant secured by the Cleveland Clinic and Cleveland Museum of Natural History Expedition to South East Africa in 1935–1936 is here described. The weights of the various subordinate parts of the body are given together with a comparison of the gland size ratios of elephant and African mouse. Thyroid gland relationships are compared in the light of the body surfaces of the two forms.

(39) *90. Some interesting human hypophyses and their functional significance.* A. T. Rasmussen, Department of Anatomy, University of Minnesota.

The morphology of some unusual human hypophyses will be shown, with a discussion of their functional bearing. More specifically, there will be included an hypophysis which has been almost doubled in size by the accumulation of colloid in the residual lumen, illustrating the lack of correlation between the amount of colloid and amount of epithelium in pars intermedia; extreme basophilic invasion of the neural lobe and absence of high blood pressure; extension of non-adenomatous anterior lobe tissue into the posterior lobe of an adult male hypophysis; hypophysis simulating that of pregnancy from a 5-year-old girl with hypergenitalism and large spongioblastoma in floor of the third ventricle; absence of castration cells in hypophysis of an adult man with no germinal epithelium, etc.

(48) *91. Topography of the ductus arteriosus in the fetus, newborn and young infant.* Irving Rehman, Departments of Anatomy, College of Dentistry and the Graduate School, New York University. (Introduced by Gustave J. Noback.)

The usual descriptions of the course, relationships and entrance of the ductus arteriosus into the aorta are not in agreement with the findings of this study. The ductus arteriosus enters the antero-lateral aspect of the descending aorta about 1 cm. distal to the origin of the subclavian artery and not, as commonly stated, at the medial border just below the origin of the left subclavian artery. The ductus arteriosus extends further inferiorly than is usually described, and lies more obliquely antero-posterior. Its course is therefore more nearly parallel with that of the aorta, from which it is separated by a small space. It joins the aorta at an angle less than the 30 to 35° commonly stated. It is described as spindle-shaped; however, many variations toward the cone and bilaterally concave types were found.

These findings and direct relationship of the thymus, left bronchus and aorta to the ductus arteriosus are of importance in formulating a hypothesis as to its physiological closure and anatomic obliteration. On the basis of these findings the topographic relationships are redescribed.

Permanent records of the several stages in dissections of fetus, stillborn and young infants were made by means of orthoscopic drawings, photographs and casts, and material illustrating the above findings may be seen in the demonstration. (See demonstration no. D45.)

(75) *92. The distention-growth response of the uterus under the influence of oestrin and progestin.* Samuel R. M. Reynolds, Department of Physiology, Long Island College of Medicine, and Willard M. Allen, Department of Obstetrics and Gynecology, The University of Rochester, School of Medicine and Dentistry.

The uteri of rabbits were chronically distended for a period of 2 weeks with suitable pellets of known size, inserted per vaginam. Some rabbits were treated first for 2 weeks with oestradiol and this treatment was continued for the period of distention. Other rabbits were treated with 6.6 rb.u. of progestin during the distention period. By means of an appropriate technic, the amount of growth at each distention site was determined and correlated with the degree of distention. In oestrinized rabbits, the capacity of the distended uterus to grow is markedly reduced, when compared to the growth in untreated, ovariectomized rabbits. The reduced growth-capacity is more pronounced in the endometrium than in the myometrium. In progestin-treated rabbits, marked distention-growth responses are obtained, if the animals are sexually mature. The amount of distention required to produce a given amount of growth was found to be much greater than in untreated ovariectomized rabbits. The basis of these effects will be mentioned.

(127) *93. Daily light ration and gonadal activity in the English sparrow, Passer domesticus Linnaeus.* A. R. Ringoen and A. Kirschbaum, Departments of Zoölogy and Anatomy, University of Minnesota.

The authors ('33, '36) have previously shown that full spermatogenic activity may be induced in male English sparrows by artificially lengthening the days (exposure to 60 or 100 watt incandescent bulb) during the fall and early winter.

In the present study male birds were capped during the exposure period to determine the importance of ocular stimulation in testicular hypertrophy. The experiment was begun on November 5th. After a 6-week period it was found that the gonads of six out of nine capped birds were spermatogenically inactive. The testes of three contained spermatocytes, but no sperms. Thirteen out of fifteen uncapped birds showed testicular enlargement, eleven of these gonads containing mature spermatozoa. Nine caged controls and two controls from nature had quiescent testes.

Female birds require a longer period of light stimulation (70 or more days if experiment is begun in early November) before significant enlargement of ovary and oviduct take place. The oviduct enlarges under the influence of secretion by the ovary of theelin or a theelin-like substance. Injections of theelin during January and early February induce precocious oviduct hypertrophy. The ovaries of such birds are small in size, compared to the enlarged ovaries of light-treated birds. (See demonstration no. D46.)

(72) *94. Changes in electrical potential during the oestrous cycle of normal and experimental rats.* Philip V. Rogers, Department of Anatomy, Yale University School of Medicine. (Introduced by Edgar Allen.)

It has previously been shown that electrical potentials, as read on the Burr-Lane microvoltmeter, show consistent changes during normal oestrous cycles in rats, and that ovariectomy and hypophysectomy cause the cyclic type of curve to disappear.

Very small, sub-minimal and oestrous-producing quantities of theelin were administered to sixty-four spayed rats. The electrical potentials of those animals receiving less than 1 international unit were of the castrate type. Larger, but not oestrous-producing, quantities caused the irregular castrate curve to flatten out. When sufficient theelin to produce oestrus was injected the potential changes formed a pattern similar to that of the normal animal.

Vaginal administration of 0.04 γ theelin causes cornification without distention of the uterus. Such animals fail to show the characteristic late oestrous rise in electrical potential.

Twenty-five hypophysectomized animals received theelin and F.S.H. (prephysin) injections. Theelin administered in three injections 8 hours apart caused the electrical potential readings to simulate those of normal animals. Remaining fragments of pituitary as small as 3 to 4% induce luteinization during or immediately after F.S.H. administration. However, if all the anterior pituitary has been removed, administration of F.S.H. causes the animals to remain in oestrus for many days even after injections have been stopped. As in normal animals a rise in potential occurs only if the animals go out of oestrus.

(73) *95. Some observations on the transport of sperm in the rat uterus.* Isadore Rossman, Department of Anatomy, The University of Chicago. (Introduced by G. W. Bartelmez.)

Using sodium amytal anaesthesia, uteri of rats were studied under a bath of flowing, warmed Ringer's solution 10 minutes or more after mating. With Knisely's quartz rod method it is easy to transilluminate the uterus and observe its contents. Under these conditions, numerous particles, up to 5 mm., are seen. Their size and number are increased after repeated matings. Peristaltic contractions are the rule; antiperistalsis or multiple contraction waves are infrequent. Usually the contracting area involves about a centimeter of the horn and appears as a progressing band; it drives the enclosed particles to the vaginal end of the uterus. This process takes 5 to 10 seconds and causes marked dilatation of the vaginal end. When the wave reaches this dilated lower end of the uterus, the sperm masses are rapidly propelled into the now relaxed tubal end. This return takes a second or two. By the use of the freezing method of Daron, these contraction waves can be fixed at any desired moment for histological study. Ninety-five per cent alcohol chilled to about — 60° is poured over the contracting viscus in situ, promptly bringing the contraction to a standstill. It is allowed to thaw in a suitable fixative and run up as usual. Slides demonstrating the plastic changes in contracting uteri and the effect of the wave on the enclosed sperm will be shown.

(19) *96. Differentiation in vitro of transverse strips of the mesectoderm of the chick blastoderm.* Dorothea Rudnick, Department of Zoölogy, University of Rochester.

The mesectoderm layer of the area pellucida of chick blastoderms of late primitive streak and early head process stages was divided into four measured segments by transverse cuts at a) the primitive pit; b) approximately one-fourth of the way back on the streak; c) approximately one-half of the way back on the streak. These strips were allowed to develop on plasma-embryonic extract clots for 2 to 10 days. Each level shows a characteristic morphogenetic capacity in vitro. The segment anterior to the pit generally separates itself into a dense vesicle or mass containing brain and epidermis, and one or more thin-walled non-axial epithelial sacs, usually empty of cellular content. The segment including the first quarter of the primitive streak develops as a compact mass containing neural tissue, chorda, epidermis, mesenchyme, and laterally, hearts which may be enclosed in vesicles. The second quarter of the primitive streak characteristically forms large thin-walled epithelial sacs, containing a little mesenchyme and a few blood cells; the median (streak) region may or may not become vesicular. The posterior half of the streak forms vesicular or condensed cell masses largely composed of erythroblasts. The above structures are all transitory, and tend to dedifferentiate to membranes on the plasma clot.

Thus it is seen that the potencies of such isolated pieces are much reduced as compared with their normal fate or with their potency when tested by other experimental methods.

(132) *97. The quantitative method in anatomical research.* Richard E. Scammon, The Graduate School and Institute of Child Welfare, University of Minnesota.

The quantitative method in anatomical research is not new, but still offers many opportunities. In general, three divisions of the method are appropriate to anatomy. The iconographic as distinct from the pictorial method arose in the sixteenth century through the efforts of clinicians, artists and anatomists. Its future development depends largely upon the application of the laws of dimensionality. The use of composite photography seems neglected, except for the work of Galton. The tabular method has been used since the eighteenth century studies of Roederer and Sue. It will always be used, but may well be reinforced by other methods.

The graphic method, including the presentation of data in analytic or quasi-analytic form, was devised by Quetelet who, by 1836, noted the analogy between the 'normal' or Laplace-Gauss curve and the distribution curve of many physical traits. Quetelet developed the first empirical formula for the post natal human growth curve. Later developments were made by Stieda (1883), Galton and others.

There are two general classes of analytic expressions, logical and empirical. Rational expressions do not seem as fruitful as empirical in human morphology, except where geometric relations are involved. The usefulness of this method has been increased through recent developments in variability measures and the invention of instruments to determine mechanically rates of change of various parts of the body.

(21) *98. The function of the egg white for the development of the chicken embryo.* Gerhard Schmidt, Queen's University, Kingston, Ontario. (Introduced by G. H. Ettinger.)

Fertilized eggs, incubated outside the shell in a mixture of egg white and a modified 'Ringer solution,' are developing during at least 4 days like eggs incubated under normal conditions. No development can be observed if the addition of egg white to the 'Ringer solution' is omitted, or if the eggs are incubated in a mixture of dialyzed egg proteins and 'Ringer solution.' Recombination of dialyzed egg proteins and the dialysate restores the original growth promoting effect of the egg white.

Since a 'Ringer solution' containing only 10% of egg white possesses still a strong growth promoting effect, it is very probable that the egg white has not only the function of a protective medium or of a food material for the later stages of embryonic life, but that it contains substances which are indispensable for the metabolism of the growing cells. The rich potassium content of egg white cannot explain the growth-promoting effect of egg white dialysate, since an increase in the potassium content of the 'Ringer solution' kills the embryo in a short time.

(79) *99. Changes in the adrenals, livers and thyroids of guinea pigs as affected by environmental temperatures and thyroxin.* Ida Genther Schmidt, Department of Anatomy, University of Cincinnati.

Sexually mature male and female guinea pigs were treated with oral thyroxin under conditions representing average room temperature and high temperatures and humidity simulating Cincinnati summers.

Adrenals vary chiefly in gross size, size of the fascicular zone, and degree of mitotic proliferation. Normal controls have few mitoses; the majority are usually in the glomerular zone. Following thyroxin treatment (total of 0.6 and 1 mg.), four to six times as many mitoses are present, mostly in the fascicular zone. Four weeks in the hot room suppresses mitotic activity, the fascicular zone is small; in 6 weeks, the mitotic activity has increased beyond that found in controls, and the gland approaches normal size. Thyroxin treatment after 4 weeks is not quite effective as in normal controls; after 6 weeks, it is still less effective.

Livers in thyroxin-treated normals remain essentially normal. Livers of hot room animals undergo a type of cell degranulation resembling fatty degeneration; cells are affected from the central veins outward. Livers of hot room thyroxin-treated animals show early fatty degeneration.

Thyroids are but slightly affected. Hot room thyroids show flattened epithelium and retracted colloid. Thyroxin-treated thyroids show distended colloid and lower epithelium than in normal controls.

These results apply to males. Females vary more in their degree of susceptibility but follow the same general trend.

(47) *100. The effects of parathyroidectomy on the incisor teeth of the albino rat and their modification following fasting or repeated pregnancies and lactations.* Isaac Schour, University of Illinois, College of Dentistry, Simon B. Chandler and Wilbur R. Tweedy, Loyola University, School of Medicine, Chicago. (To be presented by Doctor Chandler.)

This study is based on ninety-five parathyroidectomized albino rats which were operated between the ages of 21 and 111 days. The animals were divided into four groups: 1) fourteen surviving 4 to 20 days; 2) fifty-six surviving 39 to 187 days; 3) fourteen surviving 105 days and subjected to fasting each seventh day; 4) eleven surviving more than 4 months and subjected to repeated pregnancies and lactations.

The incisor teeth of the animals in group one showed disturbances in calcification and development. The post-operative dentin was hypercalcified and demarked from the pre-operative dentin by a deeply stained hematoxylin ring. In group two there was defective formation as well as defective calcification of enamel and dentin. Fasting accentuated similar histologic changes in the animals of group three but they were not so marked as in the animals of group four which survived several pregnancies and lactations. In no instance was there histologic evidence of withdrawal of calcium from the teeth.

Compared to previous reports our results show: hypercalcification in dentin in group one; less severe changes for the period of survival in group two; and a smaller amount of rachitic-like predentin in all groups. We ascribe these differences mainly to differences in diet.

(95) *101. The growth pattern, growth rhythm and ring analysis of the tooth.* Isaac Schour, Department of Histology, College of Dentistry, University of Illinois.

Three unique morphologic and physiologic characteristics permit a quantitative and qualitative analysis of the growth pattern of the tooth: 1) the rhythmic manner of its appositional growth; 2) the extreme sensitivity of the tooth to fluctuations of metabolic processes, particularly calcium metabolism, during its formative stage; 3) the permanence of tooth structure and its freedom from calcium withdrawal. Histologic examination reveals: 1) a characteristic pattern and gradient of growth in the form of incremental rings, which record the ontogenetic and chronologic history of the particular tooth; 2) a constant 16 μ incremental rhythm in these rings in the enamel and dentin of all the species studied from rat to man; 3) accentuated incremental rings of arrested growth or disturbed calcification. The neonatal ring, for example, reflects the physiologic readjustments incident to birth. Vital staining with alizarine and injections of sodium fluoride show that the 16 μ incremental rhythm is a 24-hour phenomenon in the lower species studied and a 96-hour phenomenon in the macacus rhesus monkey and man. Further findings have led to a mathematical approximation of the pattern of the incisor of the rat. Analysis of endocrine and vitamin disturbances shows that each particular dysfunction produces a characteristic record which is superposed on the basic pattern. These kymographic qualities of the tooth constitute the basis for tooth-ring analysis as an aid in the assessment of health and disease. (See demonstration no. D51.)

(57) *102. Some effects of prolonged exposure to low temperatures upon weight of organs and basal metabolism in the albino rat.* Edward L. Schwabe and Frederick E. Emery, Department of Physiology, University of Buffalo.

Studies on the effects of environmental temperature in more than 200 albino rats have been made in respect to weights of organs and basal metabolism. The kidneys, spleen, ovaries, testes, pituitary, thyroid, adrenals, brain, heart, lungs, thymus, liver, stomach and intestines were weighed immediately after the metabolism determination. The effect of low temperatures on the weights of certain of these organs will be discussed together with the changes observed in the basal metabolic rate.

Exposure to low environmental temperatures of 7 to 12°C. for 15, 30 and 60 days, respectively, increases basal oxygen consumption and heat production as measured at thermal neutrality 11 to 16%. This increase in oxygen consumption and energy output per unit of body weight reaches a maximum and sustained level of + 15% after exposure to the cold for 30 days. A 10% sex difference in energy output per square meter per 24 hours is indicated, the male rats having the higher metabolic rate. The results show a decrease in energy metabolism with advancing age. Neither the diet, weight, age or estrous cycle, nor the conditions of light, body temperature, time of day, or activity during the metabolism determinations played any part in bringing about the effects observed.

(22) *103. Tissue reactions after homoplastic and heteroplastic transplantation of eyes in the anuran amphibia.* Joseph L. Schwind, Department of Anatomy, Albany Medical College.

Heteroplastic and homoplastic eye transplants were made between larvae and embryos of Rana sylvatica, R. palustris and R. pipiens. Heteroplastic grafts between embryos differentiated normally, grew for a time but usually degenerated before metamorphosis. Heteroplastic grafts between larvae also usually degenerated before metamorphosis. All heteroplastic grafts which survived metamorphosis showed evidence of impending degeneration because the connective tissue around the eye was more or less infiltrated with leucocytes. Attempts to immunize animals against each other by double operations and by heteroplastic parabiosis were unsuccessful. Some heteroplastic transplants in R. pipiens also degenerated. The perioptic connective tissue of the cases which survived metamorphosis showed leucocytic infiltration, which is probably the beginning of degeneration. The inflammatory reaction was more severe in unrelated animals than in larvae from the same bunch of eggs. Homoplastic grafts in R. sylvatica usually appeared normal after metamorphosis, but on histological study some of them also showed infiltration of the perioptic connective tissue. The anuran amphibia resemble the higher animals rather than the urodeles in their reactions to grafted tissues. Coordinated eye movements sometimes appeared in the transplanted larval eyes within 7 days. The optic nerve occasionally regenerated following transplantation, but as a rule it did not connect with the diencephalon. All transplants made during larval life showed a great reduction in the number of cells in the ganglion cell layer of the retina.

(62) *104. Inhibition of respiratory movements from electrical stimulation of the cerebral cortex in the cat, dog and monkey* (*Macaca mulatta*). Wilbur K. Smith, Department of Anatomy, University of Rochester, School of Medicine and Dentistry.

Electrical stimulation of certain areas in the cerebral cortex of the cat, dog and monkey under light ether anesthesia, with currents of moderate strength, causes a complete cessation of breathing; weaker currents cause slowing with or without a decrease in amplitude.

In the cat and dog the most marked response is obtained from the rostral part of the gyrus compositus anterior. Cytoarchitecturally, this region in the dog belongs to Brodmann's area 6.

In the monkey the inhibitory area is situated in the lower part of the precentral gyrus just caudal to the lower end of the inferior precentral sulcus and it also belongs to area 6.

In neither cat, dog nor monkey can a complete cessation of respiration be maintained indefinitely during stimulation, for after several seconds breathing again begins but at a rate slower than that which existed prior to application of the stimulus. Under light anesthesia chewing movements are frequently obtained from the same area; a greater depth of anesthesia abolishes the chewing movements but the respiratory response remains elicitable.

An incision surrounding the inhibitory area and passing through all the cortical layers does not abolish the response; undercutting the area abolishes the response but it can be elicited by stimulation of the underlying fibers.

In the cat a pronounced rise of arterial pressure occurs simultaneously with the respiratory inhibition. This rise does not appear to be asphyxial in origin.

(2) *105. The relation of intrauterine respiration to the structural development of the fetal lung.* F. F. Snyder and Morris Rosenfeld, Department of Obstetrics and Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University.

The recent demonstration that respiratory activity is not initiated at birth but extends far back into embryonic life has prompted reexamination of the structural development of the lung of the rabbit. The pulmonary movements have been correlated with the extent of dilatation of the future air passages. By addition of India ink to the amniotic fluid a tidal flow into the lung was shown to occur in breathing fetuses but was absent in apneic ones. Structural changes occur in proportion to the amount of respiratory activity. The breathing of amniotic fluid affords a mechanism for the distention of alveoli and thinning of the septa before birth. (See demonstration no. D55.)

(112) *106. A genetic factor in development of bone.* George L. Streeter, Department of Embryology, Carnegie Institution of Washington.

Two strains of white rats have been bred, one of which is markedly vulnerable to rickets-producing diet and the other is much less so. The degree of vulnerability is determined by placing the newly weaned young on a vitamin-D-free, high calcium and low phosphate diet, for a period of 3 weeks, at the end of which time an x-ray film of the tibia is made and the disturbance in bone growth recorded. The animals are then returned to normal diet and reared, apparently uninjured by the experience. Four months later they are bred. The breeders are chosen on the basis of the x-ray record of their sensitivity to the deficient diet. After fourteen generations of inbreeding and selection, two strains have been obtained which in all appearances are alike, save in the one character that one of them following 3 weeks on the Steenbock rachitic diet during the active growing period reacts more severely than the other, and the two strains have now become homogeneous in respect to their respective reaction.

These experiments demonstrate that heredity is a definite etiological factor in abnormal bone development, of the type seen in rickets. They were made in cooperation with Dr. E. A. Park and Miss Deborah Jackson of the Department of Pediatrics of Johns Hopkins University.

(63) *107. Observations on the development of the apertures of the fourth ventricle of the human brain.* R. M. Strong and E. J. O'Donovan, Department of Anatomy, Loyola University School of Medicine.

Serial celloidin sections of the entire head for four stages, i.e., 69 mm., 84 mm., 175 mm. and 230 mm., c.r. length, were made and studied. Reconstructions of critical regions have been begun. Lantern slides made from photographs of important sections will be shown. The apertures and the lateral recesses are relatively large in these stages, especially in the youngest. Their development is intimately associated with the development of the cerebellum and the meninges.

(135) *108. Experimental human anatomy, exemplified by cutaneous innervation.* I. Maclaren Thompson, Department of Anatomy, University of Manitoba.

Gross anatomy has tended to comprise mainly description and interpretation of dead material; this has led to neglect of gross anatomy by many progressive anatomists, concerned with anatomy as a biological science. In large part, the anatomical results of experimental procedures have been scrutinized microscopically; that the macroscopic outcome of experimentation may be not less readily and fruitfully studied is indicated by such animal work as that of Evans on the growth hormone, and that of Stockard on physical types. The feasibility of experimenting anatomically upon the living human body is exemplified by the investigations of Duchenne on muscle actions, of Macklin on bronchial dynamics, of Wingate Todd on gastrointestinal levels and activities, and of Thompson and his collaborators on cutaneous nerve areas. Experimental attack upon the latter has yielded results otherwise unobtainable, such as pictures of the complete areal innervation of a certain region in one person, and quantitative data on the variability of overlaps.

By virtue of his special knowledge, the macroscopic anatomist is particularly qualified to plan wisely both the general scope and the technical details of human experimentation. Although, in comparison with animal work, experimental human anatomy is restricted by obvious considerations and difficulties, further effort in this direction seems likely to prove of value to anatomy, both as a biological science and as a part of medicine.

(113) *109. The formation of cartilage in the regenerating fore limb of larval Amblystoma after exarticulation of the humerus.* Charles S. Thornton, Department of Biology, Kenyon College. (Introduced by E. G. Butler.)

A new limb skeleton will form in regenerating fore limbs of larval Amblystoma in which the humerus had been completely removed. The cartilage of the new limb skeleton develops out of a blastema in which there are no cells of cartilage origin. This regeneration blastema is a composite structure made up of cells derived from dedifferentiation of the injured tissues of the limb. The tissues which have been observed as contributing to the regeneration blastema are: muscles, especially the muscles of the shoulder; connective tissue of the sheath of the brachial nerve plexus; muscle connective tissue; and, to a certain extent, subcutaneous connective tissue. The new cartilage of the limb skeleton develops out of this composite blastema by means of a differentiation of cells in the central axis of the blastema.

The amount of cartilage regenerated appears to depend on the mass of the blastema. In many cases the blastema fails to extend fully into the glenoid cavity of the scapula. In these cases the head of the developing humerus is deficient in size and structure. A complete limb skeleton is regenerated only when the blastema extends fully into the glenoid cavity.

Since the new cartilage of the regenerated limb has no genetic continuity with the old limb skeleton, it would appear that the limb field exerts some kind of histogenetic determining action.

(136) *110. Criteria of age changes in the growing body.* T. Wingate Todd, Western Reserve University.

Criteria of age changes in the bodily framework can be sought in the skeleton and supporting tissues, the locomotor system (muscles, tendons, joints), in the peripheral circulatory system, the skin and subcutaneous tissue and in the progressive modifications of bodily dimensions and proportions.

Evidence on all these criteria except those of the skin is most readily obtained from the skeleton which can be examined by anthropometer and roentgenogram as easily in life as it is studied by caliper and maceration after death.

The aging process in the skeleton comprises a) growth in dimensions, b) maturation changes by which the small skeleton of the embryo is transformed into the fully grown skeleton of the adult, c) modifications of texture affecting both the mobile store of mineral and the pattern of architectural features, d) the transformation of marrow areas and islands, e) the development of muscular ridges, f) changes in texture associated with modifications of vascularity, g) changes in textural strength induced by metabolic modifications of the bones, h) progressive modifications of the articular surfaces and margins.

For each of the above categories of age changes a time schedule of progress can be constructed. This is always affected and often mutilated by misadventures in constitutional health but not, so far as we have been able to ascertain, from purely special movements or posture in daily life.

(100) *111. The influence of metabolic disturbances in the eruption of teeth.* T. Wingate Todd, Western Reserve University.

This study of the dental pattern in nine sheep thyroidectomized at between 1 and 3 months of age by Doctors Simpson and Liddell of Cornell University was undertaken to ascertain the effect of constitutional imbalance in general and hypothyroidism in particular upon size, form and eruption date of teeth. Growth patterns in skull and skeleton were also studied and comparisons made with the normal twins.

The external parathyroids were left intact in each experimental animal and roentgenographic studies of the bones showed that no demineralization occurred.

The animals lived to an age, respectively, of 9, 12, 13, 14, 22, 24, 30, 32 and 59 months.

Detailed comparison of crown patterns and dimensions shows that these features are not influenced adversely by cretinism.

No caries is found in any of the experimental animals: all teeth are perfectly sound.

Comparison of tooth eruption dates with those of the controls shows retardation but a comparison of retardation in eruption with retardation in skeletal maturation of epiphyses and diaphyso-epiphysial planes make it clear that the former is affected far less than the latter.

There is simple delay in eruption up to the age of 14 months. The older sheep, however, show irregularity in eruption and it is to be noted that these animals also show, whereas the younger ones do not, frank skeletal pathology.

(64) *112. Function and structure in the chronically isolated lumbo-sacral spinal cord of the dog.* Sarah S. Tower, Department of Anatomy, Johns Hopkins University.

Could a portion of the spinal cord be isolated by cutting the pathways of incoming nerve impulses and the animal be kept alive, the following question might be answered. 1) Is such functionally isolated nervous tissue capable of surviving, and what structural alterations does it undergo? 2) Does the isolated nervous tissue exhibit activity; is the spinal cord autochthonous? 3) Is the trophic control of non-nervous tissues by the nervous system exercised by physical integrity of the nervous tissue, or does it derive from activity nervously initiated? This last question may be answered only if the nervous tissue survives but is inactive.

By sectioning posterior roots and transecting the cord above and below, all incoming nerve impulses were excluded from the lumbo-sacral spinal cord in three puppies. These were kept alive 2, 5 and 6 months thereafter. The living animals were studied for evidence of nervous activity, and the living animals, gross autopsy material and histological preparations for evidence of growth and atrophy in nervous system, skeletal muscle and bone.

The conclusions reached were: 1) that functionally isolated nervous tissue is capable of surviving; 2) that it retains the capacity for activity but is not autochthonously active; 3) that trophic control of non-nervous tissues by the nervous system involves both physical integrity of innervation and nervous activity. The evidence for these conclusions will be presented.

(53) *113. On the incidence of accessory sacro-iliac articulations.* Mildred Trotter, Department of Anatomy, Washington University, St. Louis.

Series of White and Negro skeletons examined for accessory sacro-iliac articular facets presented the variation in 50% of the White series and in 21% of the Negro series. The incidence was slightly lower in the females than in the males. An examination from the standpoint of age showed that the incidence increased with increase in age and a re-grouping of each series according to decades revealed a close agreement in each decade between the White and Negro series.

(37) *114. Pituitary acidophilia and basophilia in the German shepherd dog.* E. M. Vicari, Department of Anatomy, Cornell University Medical College, New York City.

In a study of pituitaries from eighteen German shepherd dogs, two showed wide deviations from the breed average in the quantity of differential cell types. The pituitary of a 12-year-old male showed excessive amount of acidophils throughout and markedly so in a localized extensive tumor-like mass in the central region of the pars glandular. The pars intermedia was extensively invaded with acidophils. The body weight of this individual was 99 pounds, 35 pounds in excess of the average male weight of the group. The vertebrae and especially the skull bones were thicker and heavier than these bones in the other males. This thickness in bony structures was also expressed in the limbs especially in the proximal bones. The pituitary of a 7-year-old female showed an unusual amount of large basophils and many chromophobes. Her body weight of 97 pounds due to excess adiposity was 40 pounds more than the average female body weight. The thyroids of these two abnormal individuals were less active than the breed type; they showed large distended follicles with low epithelium and uniform dense colloid.

(65) *115. Further studies of thalamo-cortical connections in the cat.* William H. Waller and Ralph W. Barris, Department of Anatomy, George Washington University Medical School.

Removals of various cortical gyri in cats cause the appearance of regions of degeneration in the dorsal thalamus demonstrating the connections of the thalamic nuclei to the cortex. Eleven experiments described in a preliminary report gave evidence of the existence of fibers from the dorsal nucleus of the lateral geniculate body to the lateral gyrus, from the medial geniculate body to the sylvian gyri, from the lateral nucleus and pulvinar to the rostral and caudal parts, respectively, of the middle suprasylvian gyrus, from the ventrolateral and arcuate nuclei to the pre- or post-cruciate gyri, and from the anteroventral and anterodorsal nuclei to the gyrus cinguli.

Subsequent experiments have corroborated these results. In addition, two nearly complete hemidecortications brought about complete degeneration of the central lateral, ventromedial, and suprageniculate nuclei, and the centrum medianum, the ventral anterior nucleus except its dorsomedial part, and some cells of the paracentral nucleus. Injuries to the frontal lobe caused complete degeneration of the medial nucleus, and chromatolysis in the anteromedial nucleus followed a lesion of the rostral half of the gyrus cinguli.

Failure to find degeneration in the tectum, in some cells of the dorsomedial part of the ventral anterior nucleus, or in any of the midline nuclei with the possible exception of the nucleus reuniens, indicates that these structures have no ascending connections to the cortex.

(8) *116. The arrangement of the cranial venous sinuses in the occipital region in man.* James Crawford Watt, Department of Anatomy, University of Toronto.

The manner in which the superior sagittal, straight, occipital and two transverse venous sinuses are united in the occipital region has been examined in 150 cases. The size and manner of anastomosis between the right and left sides was specially noted as it has a very practical significance in carrying on the circulation after thrombosis in a transverse or sigmoid sinus. Only four cases were seen in which no anastomosis was present. In three of these the superior sagittal sinus turned into the right transverse sinus and in the fourth case into the left. The superior sagittal sinus turned to the right in seventy-four cases, and to the left in twenty-eight, but in forty-eight additional cases (thirty-three inclined to the right, fifteen to the left) such a large communication existed with the other side that the two transverse sinuses were equal in size. The straight sinus was seen to fork evenly to the two sides only in four cases, but in several instances the anastomosis of the two transverse sinuses might be interpreted as an uneven forking of the straight sinus. The occipital sinus was single in 104 cases, double in forty-two, and absent in four instances. In three specimens the occipital sinus was as large as a transverse sinus and formed a main channel leading to the jugular foramina.

(27) *117. Retinal fatigue as indicated by the Feulgen-nucleal reaction.* Herman Charles Weinberg, Department of Anatomy, The University of Chicago. (Introduced by G. W. Bartelmez.)

The eyes of rabbits were dark-adapted for about 20 hours. One eye was then subjected to illumination for 10 to 25 minutes from daylight carbon arc and 'therapeutic' carbon arc, with maximum intensity at 3750 Angström units. In 3 minutes or less, after the end of the illumination, the experimental and opposite (control) eyes were fixed in the same dish and carried through the paraffin procedure together, cut in the same block, and treated by the Feulgen method.

The color reaction of the nuclei in the granular layers is much more feeble in the exposed retina. This is taken as evidence that the content of thymo-nucleic acid in the nuclei has been reduced. Various kinds of stimulation result in an increase in chromatin. These are evidences of chemical as well as morphologic change during nervous activity.

(121) *118. Some effects of crystalline androgenic materials on spermatogenesis in normal and hypophysectomized male ground squirrels.* L. J. Wells and E. T. Gomez, Departments of Anatomy and Dairy Husbandry, University of Missouri.

Forty males (Citellus tridecemlineatus) were employed in this study of the effects of Testosterone, Testosterone acetate and Androsterone (Ciba Co.). Eighteen were hypophysectomized in July when both immature and adult animals normally had aspermatic testes. Testes of all adults were inspected and measured through abdominal incisions preceding the experiment, three males being sacrificed for study. Sixteen hypophysectomized and eight normal males were injected for 30 days, daily dose varying from 0.5 to 4.0 mg., and killed together with thirteen uninjected controls on August 30th to 31st. One testis of each was weighed while fresh and prepared for microscopical study. Serial sections of the sella showed tiny fragments of pituitary in many cases but no granular cells of any type.

Testes of all controls were small and aspermatic, while those of injected males showed hypertrophy and in thirteen animals (54%) contained spermatozoa.

Testosterone caused spermatozoa production in hypophysectomized and normal males (both young and adult). Testosterone acetate inducing spermatozoa formation in both young and adult normal animals and in one hypophysectomized adult, failed to do so in immature hypophysectomized individuals. Androsterone, previously reported to cause spermatozoa production in normal animals (Wells and Moore, '36), led to spermatozoa formation in three of six hypophysectomized males.

We feel that we must do additional experiments to determine whether these substances can induce spermatozoa formation in complete absence of pituitary.

(56) *119. Measurements and observations upon the effect of diet on the intestine of the rat.* J. L. Wierda, Department of Anatomy, University of Pennsylvania. (Introduced by E. R. Clark.)

The data presented are introductory to an investigation into the factors which regulate the growth and determine the length of the intestinal canal. In this preliminary work the effect of unbalanced, artificial diets upon the dimensions of the rat was studied. Also the effect of malnutrition upon body size and intestine length was observed. In addition, various kinds of proteins were used to alter the quality of the diet, and their effect was recorded. The mucous surface of the intestine was photographed so that the response of the villi to the diets could be shown.

(106) *120. Preliminary observations of thyroid tissue implanted in a transparent chamber installed in the rabbit's ear.* Roy G. Williams, Department of Anatomy, University of Pennsylvania.

Autogenous grafts of thyroid gland were implanted in a transparent chamber installed in a rabbit's ear. Two types of follicles were observed—one which was small with a relatively thick wall and containing one or two vacuoles, another which was larger, with thinner walls and a larger number of vacuoles.

The implant was observed daily for 30 days. Three injections of a stock preparation of antuitrin were then given subcutaneously on successive days: 1 cc. the first day, 2 cc. the second day, 5.5 cc. on the third day. These produced a swelling of the entire follicle, walls and colloid, followed by a sudden but small drop with the last dose. Three days after the last dose there was some increase in thickness of the follicular wall and decrease in colloid. This was followed by a shrinkage of wall area and an increase in area of colloid. Six days after the last of the previous injections, three doses of thyroprotein, 2 cc. each, were given subcutaneously on successive days. These injections produced a further diminution in follicular wall area and an increase in colloid. Six days after the last injection the graft was fixed together with a sample of thyroid tissue from the neck of the same animal. The sections of the graft were similar to those from the control and were regarded as being normal.

(89) *121. On the localization of heart-forming areas in chick blastoderms of pre-somite stages.* B. H. Willier, Department of Zoölogy, The University of Rochester.

An attempt has been made to locate the heart-forming areas in chick blastoderms of pre-somite stages by testing the capacity of certain isolated and accurately measured segments to develop cardiac muscle in chorio-allantoic grafts. In the head-process stages, which have been studied most intensively, these are two separate elongated areas lying approximately 0.2 mm. to either side of the mid-line, extending from a level 0.5 mm. anterior to 0.35 mm. posterior to the primitive pit, approaching a millimeter in total length. The outermost lateral boundaries have not been too well defined due to the low graft frequency exhibited by lateral pieces cut more than 0.4 mm. to either side of the mid-line. Evidence indicates that the areas are slightly further apart posteriorly than anteriorly.

Heart tissue develops with the highest frequency (90% or more) at the node level, declining from here in both directions, more gradually anteriorly than posteriorly. Although graft frequency is noticeably lower on the right side of the blastoderm, the heart tissue which differentiates from the right pieces is in no respect inferior in quality and quantity to that developed from similarly isolated left pieces. The heart is a rounded or oval-shaped mass of cardiac muscle without chambers. It is typically differentiated and occasionally exhibits strong pulsations. Apparently there is no correlation between pulsation and degree of differentiation.

At the definitive primitive streak stage the heart areas are less elongate and nearer to the mid-line (as close as 0.1 mm.). Pre-streak stages give heart from the posterior portion only of the area pellucida where the primitive streak arises.

(1) *122. Some factors governing initiation of respiration.* William F. Windle, Anatomical Laboratory, Northwestern University Medical School.

Rhythmic respiratory movements appeared normally about 3 days before chicks hatched. Integrated head and trunk movements of 6- to 10-day chicks became incorporated in the respiratory pattern.

During the last half of incubation, complete anoxemia was followed by a latent period of 1 or 2 minutes, then occurred quickening of all activity, depression of non-respiratory movements, loss of irritability, gasping and rhythmic respiration. These events appeared with less regularity in partial anoxemia; impairment of heart action was a predisposing factor.

All activity quickened and rhythmic respiratory movements began in atmospheres containing 30 to 45% oxygen and 0.8 to 1.6% carbon dioxide. With 1.6 to 5% carbon dioxide, non-respiratory movements were depressed and respiration became deeper. With 7 to 10%, deep, tonic, rhythmic gasping alone persisted. There was no latent period comparable with that following establishment of a state of anoxemia. (See demonstration no. D60.)

(104) *123. Studies on the islets of Langerhans after continuous intravenous injection of dextrose and insulin.* Arthur Woerner, Department of Anatomy, The University of Chicago. (Introduced by R. R. Bensley.)

Guinea pigs were injected with 1) a solution of dextrose, 2) insulin and 3) insulin with dextrose, for varying periods of time. Determinations of blood sugar were made on controls and on several of the experimental animals during the course of the experiment.

Some of the animals of each series were perfused with Janus green and whole mounts made of the pancreases. The pancreases of the others in each series were fixed by formol-Zenker, acetic-osmic-bichromate and Regaud, sections of which were stained with aniline acid fuchsin—methyl green, neutral gentian, Altmann's and Heidenhain's modification of Mallory. The methods and results of these experiments are described.

(42) *124. Action of testosterone and related compounds on the anterior hypophysis.* J. M. Wolfe and J. B. Hamilton, Departments of Anatomy, Vanderbilt University School of Medicine, and Albany Medical College and Union University.

Immediately following operation, twenty castrated immature rats received ten daily injections of either 500 γ of testosterone, testosterone-propionate (Schering) or testosterone-acetate (Ciba) in peanut oil. Twenty-three castrated littermate controls were used. In the latter the basophiles were markedly enlarged and increased to a mean level of 14.7%; a majority were granular. In the injected rats such castration changes were prevented; the basophile level was low and a majority were degranulated. Such injections failed to induce any constant changes in the eosinophiles; the levels of these cells were practically the same in both groups. Three groups of ten mature female rats received ten daily injections of 500 γ of testosterone-acetate, 500 γ of oestrone, and a combination of these two factors, respectively. Injection of the male factor simultaneously with oestrone either partially or almost completely suppressed the pituitary response to oestrone, i.e., weight increase, degranulation and decrease in percentages of the chromophiles and an increase in chromophobes. Similar results were obtained using castrated female rats. Previous findings indicate that injection of large amounts of A.P.L. (Follutein-Squibb) simultaneously with dihydroestrin-benzoate, in either normal male or female rats, partially suppressed the action of oestrin on the pituitary in the same way as does the male factor. A.P.L. exerted no effect on the pituitary response to oestrin in castrated rats.

(25) *125. Self-differentiation of heterotopic ear ectoderm in the embryo of Amblystoma punctatum.* C. L. Yntema, Cornell University Medical College, New York City.

Stages in differentiation of the ear ectoderm were studied in the embryo of Amblystoma punctatum. Grafts containing the ear ectoderm from embryos at the yolk plug stage (stage 12) and at later stages were placed anterior to the ear region and in the forelimb region of embryos in the early head process stage (stage 22).

Ear ectoderm from a donor as young as stage 12 may form a simple ear vesicle in the region anterior to the ear. The donor must be older than stage 18 before its ear ectoderm is self-differentiating in the limb region in a majority of experiments. The normality of the heterotopic vesicles in a given region is directly correlated with the ages of the donors.

The results indicate that the ear ectoderm is differentiated from surrounding ectoderm by the end of gastrulation and subsequent differentiation continues throughout the stages studied.

(70) *126. Correlated anatomical and physiological studies on the developing legs of anuran tadpoles.* Karl Youngstrom, University of Kansas. (Introduced by H. C. Tracy.)

Development of motility in several anuran species was studied. In Pseudacris triseriata development of motility of the hind leg passes through the following stages of development: 1) Non-motile stage; mechanical stimulation of leg may result in swimming movements of the body but the leg remains passive. 2) Stage of spontaneous movement; underlying or more distal muscles respond to light touch during a temporary brief period; this response may be correlated with the extreme pliability of the skin and softness of the tissues; epidermis two cells thick, underlaid by numerous argentophilic nerve fibers. 3) Stage of response to strong mechanical stimulation; response to deep poke is diffuse, with no regular pattern; light touch reaction apparently disappears. 4) Stage of definitive light touch response; independent withdrawal reflex of leg to light touch gradually acquired; epidermis three cell layers, invaded by nerve endings, basal layer columnar.

Stages 2 to 4 inclusive occupy 1 to 2 weeks. The arms become motile 3 to 5 days before the legs, but development of light touch response lags in the arms.

The earliest movement of the leg (stage 2) is apparently dominated by body movements which can be elicited much earlier, even before the leg has appeared; however, discrete spontaneous movements of the leg may occur in this stage. Later (stages 3 and 4), leg gradually acquires independent exteroceptive response of definite pattern.

(144) *127. Micro-manipulative operations on blood capillaries of the frog.* Benjamin W. Zweifach and Robert Chambers, Washington Square College, New York University.

The use of mechanically controlled microneedles has made it possible to reveal certain features, concerning blood capillaries and their cellular constituents, which are difficult or impossible to demonstrate by other means in the living state. The motion picture shows various configurations of the capillary bed, including the so-called arteriole-venule bridges, the structure of the capillary wall and the distribution of the different cellular elements related to it.

Endothelial cells are made to contract individually by localized prodding. There is no evidence for endothelial desquamation, or for capillary dilatation and contraction induced by cells other than the endothelial cells themselves. Mechanical irritation produces a stickiness of both the endothelial wall and of the intravascular leucocytes. Recovery and disruption of the resulting cellular plug can be followed under high magnifications.

The introduction of starch granules into the surrounding tissue causes leucocytes to adhere to the capillary and to diapedese. There has also been recorded reflex capillary dilatation and peculiar reactions in the capillary during stasis.

DEMONSTRATIONS

D1. Enlarged hypophyses in old albino rats, microscopic sections and photographs. W. H. F. Addison, Department of Anatomy, University of Pennsylvania School of Medicine. (No abstract.)

D2. Quantitative studies on the human kidney. Raymond B. Allen and Richard E. Scammon, School of Medicine, Wayne University and Graduate School and Institute of Child Welfare, University of Minnesota.

The prenatal and postnatal growth of the kidney has been studied quantitatively. The weight of both kidneys with respect to fetal body length (958 observations) is a function of body length raised to nearly the third power. The relation of kidney to fetal body weight (1209 observations) follows a straight line, except for the 1000 to 2500 gm. intervals where kidney weight is greater than might be predicted. Relative kidney weight is greatest about the eighth fetal month. There seems to be a smaller rise in early childhood.

There is a sex difference in kidney weight after birth (3848 observations), except between 5 and 10 years. The coefficient of variation, like that of most major human viscera, is about 25 to 30%. The organ follows a typical double S-shaped growth curve.

The volume of medulla and cortex was determined by graphic reconstruction: the single sections being questionable for determining this relationship. Both cortical and medullary growth are rapid in the latter lunar months. The postnatal medullary growth is less rapid; the cortex increases rapidly. There is little change in the cortex-medulla ratio after early childhood.

There seem to be two periods of intense renal growth, one in the middle of prenatal life (possibly representing hyperplasia of the renal tubules) and one in early infancy (possibly representing hyperplasia of the tubules).

D3. The early development of the sensory system in albino rat fetuses. A. W. Angulo y Gonzalez, The Wistar Institute of Anatomy and Biology.

Histological material prepared by the silver technique, photographs and drawings will be offered to illustrate the early development of the sensory system in albino rat fetuses. (See also abstract no. 6 and demonstration no. D4.)

D4. Neurological interpretation of fetal behavior. The development of activity in albino rat fetuses. A. W. Angulo y Gonzalez, The Wistar Institute of Anatomy and Biology. (Motion picture.)

This record of the development of behavior in albino rat fetuses is offered to illustrate the following:

At 378 hours the only reflexogenous zone in the fetuses from which true reflexes can be elicited is the snout region. As the fetuses progress in development, responses can be elicited from other regions of the body until a period is reached in which the body surface in its totality serves as a reflexogenous zone. When this occurs there is a complete lack of regional discrimination in the animal, and the responses elicited are identical irrespective of the zone or source of stimulation. It is not until the fetuses have reached the latter part of gestation that regional discrimination begins to emerge within that generalized field of receptivity and the responses begin to be specific in relation to the zone of stimulation. (See also abstract no. 6 and demonstration no. D3.)

D5. Evidence against the existence of centrifugal fibers in the optic nerve of mammals. L. B. Arey and H. V. Smith, Department of Anatomy, Northwestern University. (See abstract no. 8.)

D6. Drawings, photographs and preparations illustrating the form and relations of the premaxilla in man. M. F. Ashley-Montagu, Departments of Anatomy, College of Dentistry and the Graduate School, New York University. (Introduced by Gustave J. Noback.) (See abstract no. 10.)

D7. Specimens illustrating the masticatory apparatus as an inductor of growth in skull bones. Lawrence W. Baker, Harvard Dental School. (Introduced by T. Wingate Todd.) (No abstract.) (Illustrating round table.)

D8. A model showing the utriculo-endolymphatic valve and neighboring structures. T. H. Bast, Department of Anatomy, University of Wisconsin. (See abstract no. 11.)

D9. Development of lymphocytes into myelocytes in tissue culture. William Bloom, Department of Anatomy, The University of Chicago. (See abstract no. 12.)

D10. Staining of nerve endings in paraffin sections with activated protargol. David Bodian, Department of Anatomy, The University of Chicago. (Introduced by G. W. Bartelmez.)

By the use of proper fixatives preceding impregnation with activated protargol, it is possible to stain selectively many diverse elements of nervous tissues. It has been found that by using a single impregnation technique, practically all of the selectivity of the stain can be made to reside in the initial fixation, with extremely constant results. Preparations of nerve fiber endings in muscle, in the pars intermedia of the hypophysis, in the adrenal medulla, in taste buds, in the vestibular sense organs, and in the central nervous system are presented. In the central nervous system, nerve fiber endings, for example the synapses on Mauthner's cell in the goldfish, can be made to show either protoplasmic and neurofibrillar continuity or discontinuity at the synaptic junction, simply by modifying the initial fixation while keeping the impregnation technique constant. In this method, where the actual impregnation process is apparently progressive and self-limited, and thus can be almost completely controlled, the variables introduced by the properties of the fixing agents used prevent a critical solution of the synapse problem. It appears, then, that the exclusive use of silver impregnation methods cannot be considered critical in any study of the finer structure of the synapse.

D11. Microscopic preparations of the pancreas of a diabetic dog before and after treatment with insulin. Donald J. Bowie, Department of Histology and Embryology, McGill University, Montreal.

A dog was made diabetic by partial pancreatectomy. Three subsequent operations were performed on the same animal to remove small pieces of pancreas for microscopic examination. When a maximal degree of hydropic degeneration was exhibited in the beta cells of the islands of Langerhans the diet was changed and insulin was given. After 5 weeks of treatment with insulin the island cells show no hydropic degeneration and appear to have returned to a normal condition.

D12. Observations on the secretory granules in fixed preparations of human gastric glands. Donald J. Bowie, Department of Histology and Embryology, McGill University, Montreal. (See abstract no. 16.)

D13. A human embryo in the bilaminer blastodisc stage (the Edwards-Jones-Brewer ovum). John I. Brewer, Department of Anatomy, The University of Chicago and Henry Baird Favill Laboratory, St. Luke's Hospital, Chicago. (Introduced by G. W. Bartelmez.)

Serial photomicrographs and reconstructions of the normal, perfectly fixed and stained embryo are presented. The closed amniotic cavity measures 0.223 × 0.178 × 0.167 mm. The yolk sac is lined by squamous cells and measures 0.197 × 0.160 × 0.120 mm. Throughout the broad attachment of the amnion and the chorion the mesoderm is continuous. There is a condensation in this mesenchyme caudally indicating the differentiation of the body stalk. There are no blood vessels or blood cell formations in the body stalk or yolk sac.

The symmetrical blastodisc measures 0.209 × 0.177 mm. The tall columnar cells of the primitive ectoderm rest upon a basement membrane. The underlying entodermal cells of the disc are cuboidal. In the five caudal-most sections of the disc the basement membrane is lacking; the two-cell types intermingle; and there is evidence of active proliferation and migration of the cells from the upper layer. This together with the early body stalk development indicates the embryonic axis. It represents the earliest primitive streak formation so far described for man.

D14. Preparations and experiments illustrating the physical liquid-pressure nature of the nerve impulse and the physical nature of muscle striations. Eben J. Carey, Department of Anatomy, Marquette University School of Medicine.

a) Sections of nerves from rabbits killed with strychnine showing constrictions of the axon where it is stretched alternating at intervals of 15 to 75 μ with dilatations where pressure pulses are fixed in place. The pressure pulses are composed of striae 5 to 10 μ in length. Large pulses of the axon are in direct contact with the neurilemma, myelin overlying only the constrictions of the axon. Schmidt-Lanterman lines are adhesion bands between axon and neurilemma, and are found only in relatively quiescent or in greatly stretched portions of the nerve. b) Sections taken after action of anaesthetic drugs and depressant environmental conditions, showing reduced histological signs of pressure pulses. c) Photomicrographs of nerve spectrum of radiant energy due to liquid pressure waves. d) Sections showing direct evidence of the liquid nature of the axon, by expression of drops from fresh nerves. e) Experiments and sections illustrating transformation of smooth tubular muscle into cross striated muscle in the leech. f) Mechanical model of cross striated muscle or nerve fiber showing reciprocal action of external tension and internal pressure, in the production of alternating energy phases and striations. g) Photomicrographs of frozen section of striated insect muscle, showing that the terminations of the air system within the muscle fiber are air tubules and not solid or liquid filled. (See abstract no. 22.)

D15. Regional potencies in the hind limb bud of the 72-hour chick. Stanley H. Chambers, Department of Biology, McMaster University. (Introduced by A. Emerson Warren.)

Previous investigators have shown that the limb buds of chick embryos are mosaic systems. The purpose of the present experiments is to demonstrate the potencies for limb formation resident in specific regions of the hind limb bud of the 72-hour embryo. The development of limbs following the extirpation of various portions of the bud has been observed. The resulting skeletal features of the limb have been indicated by the alizarin red S staining method. A number of cases will be exhibited.

D16. Nerve fiber terminations in the intrinsic muscles of the eye. Kermit Christensen and W. A. Stotler, Department of Microanatomy, St. Louis University School of Medicine.

The preparations show the terminal structure of the nerve fibers in the ciliary muscles and the muscles of the iris. Some are taken from normally innervated eyes; others from eyes in which either the sympathetic or the parasympathetic fibers have undergone degeneration.

D17. Cyclic changes in lymphatic tissue. Eleanor A. Conway, Department of Anatomy, The University of Chicago. (Introduced by William Bloom.) (See abstract no. 25.)

D18. Sections illustrating correspondences in the lateral cortex in some reptiles and birds. E. Horne Craigie, Department of Biology, University of Toronto. (No abstract.)

D19. Interaction of hormones and genotypes in pheasants. C. H. Danforth, Department of Anatomy, Stanford University.

The demonstrations will show feathers representing the products of various combinations of genetic and endocrine factors in several species of pheasants. The specimens are thought to indicate that within the normal range of hormonal concentrations male and female genotypes show quite different reactions, but with extreme deviations from normal the two responses become approximately the same. This suggests that the difference between the ZZ and WZ chromosomal complexes, which determine the sexes, is fundamentally a quantitative one. But while the physiological basis for the different reactions is probably quantitative, the ultimate structural manifestations are expressed in differences of a qualitative nature.

D20. The vagus nerve distal to the nodose ganglion of the cat. Franklin S. DuBois and James O. Foley, Department of Anatomy, University of Alabama.

Transverse sections of the normal vagus nerve distal to the nodose ganglion prepared by various refined silver techniques demonstrate black dots so fine that they defy enumeration by ordinary methods. Since similar dots are seen in nerves from which efferent fibers have been eliminated, it is believed that they have some relation with sensory fibers. Surprisingly, these minute elements are much smaller than the smallest unmyelinated fibers previously observed in the vagus proximal to the nodose ganglion, an observation at variance with the well-known fact that peripheral processes from cells of a sensory ganglion are larger than central processes. Accordingly, they must be artifacts, supporting tissues, neurofibrillae or branches of axons. Because of their appearance, distribution and arrangement, the last interpretation is tentatively favored and if such proves to be true, the number of fibers in the vagus distal to the nodose ganglion exceeds that proximal by a far greater margin than has hitherto been suggested or anticipated. (See also abstract no. 36 and demonstration no. D22.)

D21. The subarachnoid space and the nasal mucosa. William M. Faber and O. A. Mortensen, Department of Anatomy, University of Wisconsin.

A number of substances have been introduced into the cranial and subarachnoid space of living animals and identified after a short interval in the nasal mucosa and cervical lymphatics. This demonstration illustrates the pathway of thorotrast (colloidal Tho_2) along the perineural spaces of the olfactory nerve filaments and the distribution of the thorotrast in the nasal mucosa.

It has also been demonstrated (Clark, '29) that an aqueous solution of potassium ferrocyanide and iron ammonium citrate will penetrate the living nasal mucosa of the rabbit and ascend to the subarachnoid space around the olfactory bulbs. Our material indicates the essential identity of the route whether the foreign materials are passing in an ascending or a descending direction.

This subject is of practical interest because the pathways illustrated are identical with those along which the virus of equine encephalomyelitis and pyogenic bacteria in certain cases are believed to invade the cranial cavity.

D22. Histological preparations of the vagus and auricular nerves of the cat. James O. Foley and Franklin S. DuBois, Department of Anatomy, University of Alabama.

Pyridine silver and osmic acid preparations of the vagus nerve immediately distal to the jugular ganglion showing the distribution of jugular fibers following section of the vago-accessory roots and removal of the nodose ganglion. The jugular fibers are few in number and are disposed as a group in a limited part of the cross sectional area of the vagus.

Pyridine silver and osmic acid preparations of the auricular nerve. The auricular branch of the vagus resembles a typical cutaneous nerve in that it consists of myelinated fibers 1.5 to 10.5 μ in diameter among the interstices of which are large numbers of unmyelinated fibers, singly or in clumps. The auricular contains approximately one-fourth of all the sensory fibers found in the proximal portion of the vagus nerve. (See also abstract no. 36 and demonstration no. D20.)

D23. Cytological studies of the anterior pituitary of the rabbit before and after its physiological stimulation. H. B. Friedgood and Alden B. Dawson, Department of Physiology, Harvard Medical School and the Biological Laboratories, Harvard University. (To be presented by Doctor Dawson.) (See abstract no. 39.)

D24. Duodenal diverticula. J. C. B. Grant, Department of Anatomy, University of Toronto.

Of 176 duodena filled under gravity with paraffin wax twenty were found to have diverticula. Four out of sixty-three were from subjects under 50 years of age (the youngest with a diverticulum was aged 40); sixteen out of 113 were over 50 years of age. In sixteen specimens the diverticula were single, in three double, and one had three diverticula. Only the second and third parts of the duodenum were involved.

D25. Histological changes in growing bones produced by various experimental metabolic disturbances. Arthur Ham, Department of Anatomy, University of Toronto.

Metabolic disturbances affect the growth of bones by either a) interfering with the normal proliferation of cells and their continued production of the organic components of matrix or b) interfering with the calcification of formed organic matrix. Vitamin C deficiency is shown to produce a lesion which is an example of (a), the deficiency interfering with the proliferation of cells and so with the continued formation of the organic portion of matrix. This kind of lesion is seen to differ from those of (b) which are shown to be produced by interfering with any one of the several steps in the complicated process by which calcium and phosphorus, ingested in the diet, is successfully deposited in bone. Examples of bones affected by disturbing absorption, transport or state of calcium and phosphorus are illustrated by bones taken from animals subjected to improper calcium phosphorus ratios, vitamin D deficiency, vitamin D over dosage, parathyroidectomy, parathyroid hormone over dosage and acidosis.

D26. Axis determination in limb primordia of chick embryos. Viktor Hamburger, Department of Zoölogy, Washington University, St. Louis. (Introduced by R. J. Terry.)

Hind limb and wing primordia of chick embryos with 28 to 35 somites (54 to 68 hours of incubation) were transplanted to the flank of embryos of the same stages. The primordia of the earliest stage used were thickened ridges of the somato pleura, those of the older stages were very small bud-like elevations.

The anterior-posterior axis and the dorso-ventral axis were reversed, either by transplanting left primordia to the right side or by rotating the right primordia 180°. All the twenty-six positive cases showed self-differentiation of the transplants with respect to both axes. In both wing and hind limb primordia, the axes are definitely determined at the stage when the primordia first become visible.

D27. The innervation of chromaffin tissue and the regeneration of nerves to the adrenal. W. H. Hollinshead, Department of Anatomy, Duke University School of Medicine.

Bilateral section of the rami communicantes of the upper lumbar sympathetic chains, with interruption of the chains at the diaphragm, has resulted in degeneration of the majority of the nerve fibers in the abdominal (extra-adrenal) chromaffin tissue of the cat and dog. Bilateral section of both roots of the lower thoracis and upper lumbar spinal nerves, the dorsal roots being sectioned between their ganglia and the spinal cord, has also produced degeneration of the nerve fibers in this tissue. From these experiments it appears probable that the abdominal paraganglia have a preganglionic innervation similar to that of the adrenal medulla.

The regeneration of nerves to the adrenal was studied in cats which had undergone a unilateral thoraco-lumbar chain ganglionectomy. Little evidence of regeneration of nerves to the medulla was found before the third month after operation; the medullary plexus was reforming in animals killed after 3 months, and was approximately normal in two of the three animals killed after 4 months. Observations and experiments indicated that the regenerating fibers arose from lower thoracic and upper lumbar spinal nerves.

D28. Microscopic and gross preparations demonstrating the comparative rates of absorption of cow horn, ivory, beef bone and autogenous pegs inserted into bone cortex. Charles W. Hughes, Department of Anatomy, Loyola University School of Medicine. (Introduced by R. M. Strong.) (See abstract no. 57.)

D29. Experimental study on the histogenesis of the sympathetic trunks in the chick embryo. David S. Jones, Department of Anatomy, University of Minnesota. (Introduced by E. A. Boyden.)

A demonstration of slides and wax reconstructions. (See abstract no. 64.)

D30. Developmental stages of the 'pernicious anemia neutrophil' in biopsied bone marrow. Oliver P. Jones, Department of Anatomy, University of Minnesota. (Introduced by Hal Downey.)

Studies of dry imprints of biopsied pernicious anemia marrow revealed that neutrophils in this disease have an entirely different ontogeny from those in normal marrow. There is a precocious nuclear polymorphism in the leukoblast and promyclocyte stages which is manifested by complex and bizarre configurations. There is, however, little or no change in the chromatin network. Nuclear development and maturation may precede that of the cytoplasm to the extent that during the promyelocyte stage some cells might be likened to a giant 'stab' form. Early neutrophilic granules are very fine and stain metachromatically. Later these become larger and more acidophilic than normal. There is a fairly constant cytoplasmic vacuolization which gradually disappears with cytoplasmic maturation. Perforations of the nucleus are also found. Some myelocytes may have a single large nuclear perforation which gives them the appearance of a torus.

Other pathologic neutrophils may have two or more irregular holes in the nucleus, each being separated by a chromatin strand of varying thickness. It is by means of these holes in the nuclear material that the pathologic neutrophil ultimately attains the morphology of macropolycytes (II and III) described by Cooke. Our interpretation of these alterations is that they represent processes in the maturation and development of a pathologic neutrophilic series and not degeneration (contra Tempka and Braun). These pathologic neutrophils are not related to megakaryocytes.

D31. Anatomical and physiological studies on the thyroid gland of the embryo albino rat. Helen W. Kaan and Ada R. Hall, Department of Zoölogy and Physiology, Wellesley College.

The earliest colloid to appear in the developing follicles is of the alkaline type. Acid colloid can be seen definitely at 19 days of gestation. Thyroid glands were ground up in saline solution and injected into groups of frog tadpoles. No reaction followed injection of 16- or 17-day thyroids. Tadpoles injected with thyroids of embryos from 18 days to full term showed distinct indications of metamorphosis—leg growth and lung development. The amount of colloid (per cent of total gland) is being determined for litter mates of the embryos used for the injections. From these data, cubic millimeters of colloid injected at each age can be calculated.

D32. The red blood cells of mammalian embryos. Arthur Kirschbaum, Department of Anatomy, University of Minnesota. (Introduced by Hal Downey.)

Comparison is made of the appearance of similar blood cells in sections and dry imprints of 14-day rabbit embryonic liver. Mature and immature stages of the yolk sac megaloblast are compared with like stages in the development of normoblastic red cells of the embryonic liver. (See also abstract no. 67.)

D33. Motion picture of pinocytosis by malignant sarcoma cells. Warren H. Lewis, Department of Embryology, Carnegie Institution of Washington.

Many malignant sarcoma cells from rat and mouse tumors often show in tissue cultures active wavy ruffle pseudopodia which engulf complex fluid media containing proteins and other substances that cannot diffuse into them. The fluid enters the cells as globules which move centrally. The contents are digested and the fluid then diffuses out of the cell. Thus many sarcoma cells may at times exhibit considerable intracellular digestion. Pinocytosis by malignant cells is similar to pinocytosis by normal macrophages.

D34. Epicytes from lung of cat insufflated in vivo with OsO_4. Charles C. Macklin. University of Western Ontario. (See abstract no. 73.)

D35. Histological material from hydrops fetalis. Madge Thurlow Macklin, University of Western Ontario. (Introduced by Charles C. Macklin.) (See abstract no. 74.)

D36. Demonstration of simultaneous infra red and roentgen photography: Crystallography with polarized light. Leo Massopust, Department of Anatomy, Marquette University School of Medicine. (Introduced by E. J. Carey.) (No abstract.)

D37. Studies on the human submaxillary and the sublingual glands and their relation to the attachment of the mylohyoid muscle. Shirley P. Miller and Harold L. Harris, Department of Anatomy, University of Minnesota.

A series of orthoscopic drawings from fifty specimens. These illustrate the variation in size and shape of the glands. If the sublingual gland is large, the line of attachment of the mylohyoid muscle dips downward making a distinct curve of the mylohyoid line. As a rule if the submaxillary gland is large, the sublingual gland is small and vice versa. The relation of the lingual nerve to the submaxillary duct appears to be very constant. In only one instance of the 100 glands studied was the relation reversed.

D38. The morphology of the mammalian ovary with particular reference to the follicles, theca interna, interstitial tissue and corpora lutea. H. W. Mossman, Department of Anatomy, University of Wisconsin.

The mammalian ovaries exhibited indicate that: 1) The follicular epithelium is homologous to the coelomic epithelium, the antrum to the coelom, and the liquor to the coelomic fluid. 2) Cytologically and histologically theca interna is typical endocrine gland tissue. It arises from stroma adjacent to the granulosa, is functionally best developed at estrum, and either degenerates or changes into interstitial tissue soon after ovulation. Only theca interna of large normal or large atretic follicles differentiates functionally. The latter form 'corpora lutea atretica' which contain no lutein cells and are really thecal glands. 3) Interstitial cells usually form from stroma cells adjacent to atretic primary and secondary follicles, also from immature thecal gland cells of younger graffian follicles, and possibly to a small extent from formerly functional thecal gland cells of ruptured follicles and large atretic follicles. 4) Lutein cells originate from granulosa cells of ruptured follicles. Rarely in certain monovulatory and diovulatory species (porcupine and agouti) having one large corpus luteum per embryo, numerous small corpora of like age occur, indicating the probability that immature unruptured follicles can give rise to true lutein bodies. Thecal gland cells often persist as an irregular outer zone around young corpora lutea during approximately the period of tubal migration of the ova. 5) The only distinctly glandular elements of the mammalian ovary are theca interna, interstitial and lutein tissue.

D39. Orthoscopic drawings, photographs and casts of the anatomy of the thorax of the fetus, newborn and infant. Gustave J. Noback, Departments of Anatomy, College of Dentistry and the Graduate School, New York University. (Illustrating round table; no abstract.)

D40. Nerve endings impregnated with the chloral hydrate-alcohol ammonia formula of the method of Cajal. José F. Nonidez, Cornell University Medical College, New York City.

The excellent results obtained with this silver impregnation formula in the staining of nerve endings will be demonstrated. Preparations will be shown of pericellular baskets in cardiac ganglia, pressoreceptor nerve endings, and climbing fibers in the cerebellum.

D41. Experimental studies on the cartilage plate in the long bones of the rat. R. F. Nunnemacher, Biological Laboratories, Harvard University. (Introduced by Alden B. Dawson.)

Direct amputation of the cartilage plate results only rarely in complete regeneration. More frequently cartilage-free repair, isolated cartilage nodules or 'caps,' or incomplete active cartilage plates are found. The factors of age and level of amputation are important. Amputation directly proximal to the cartilage plate before the twenty-first day in plates of lapsed-union (distal femur) are most favorable. In the typically closing plates (proximal radius, distal tibia) the restriction of regeneration capacity occurs earlier. In partial amputation youth as well as close amputation favors the regeneration of a new half cartilage from the cut edge of the old plate. A reversed cartilage plate retains its polarity and an injured (fractured or transected) plate 'closes' early. Auto- and homoiotransplantations of the ends of limb bones were successful in immature animals where a good blood supply was readily available, as in the abdominal cavity or brain case. Such transplants may show development and differentiation from unossified cartilage elements into typical bones. Growth may be almost comparable to the normal and growth without ossification may occur. Closure of the cartilage plate may be greatly retarded. Transplants into older animals generally succumb due to fibrocytic infiltration. Polarity of mitosis in cells of the cartilage plate freed from mechanical pressure by transplantation is frequently atypical.

D42. The thalamus of a dog without a hemisphere. James W. Papez, Department of Anatomy, Cornell University, Ithaca.

The left hemisphere and the cephalic part of the corpus striatum were absent due to congenital agenesia. The medial, the anterior and the lamellar nuclei were present though reduced. The medial nucleus was afferented via the lamellar nuclei. The anterior nucleus was afferented via the mammillothalamic tract. These nuclei send an inferior peduncle toward pallidal rudiments and an anterior radiation toward a remnant of frontal cortex. The ventral lateral nuclei (neothalamus) were represented by genetic rudiments resembling those in the reptilian thalamus. Reduced ascending pathways give rise to neuropil in these nuclei. The optic tract, as in reptiles, was related to pars ventralis of the lateral geniculate, pretectal nucleus and tectum. The pars ventralis thalami was nearly normal. The ventral supraoptic decussations were present, arising from the ventral parts of the geniculate bodies.

An entopeduncular nucleus was present. It corresponded to the medial segment of the globus pallidus, since the inferior peduncle ended in it, and since a small fasciculus lenticularis and the anterior hypothalamic decussation arose from it. It obviously occupies a diencephalic position. The lateral segment of the pallidum, the putamen, most of the caudate nucleus and amygdala were absent. Both medial and lateral forebrain bundles were present as in lower forms. The internal capsule and basis pedunculi were practically absent. The hypothalamus and epithalamus were normal. A rudimentary fornix was present.

D43. The cultivation of large quantities of adult tissue in fluid media. Raymond C. Parker, The Rockefeller Institute, New York.

A demonstration of the essential features of a technique for maintaining adult tissues in a state of functional survival without proliferation of the constituent elements. (See also abstract no. 85.)

D44. The Dioxan-paraffin technic for making sections of amphibian ova and embryos. William O. Puckett, Laboratory of Comparative Anatomy, Princeton University.

Microscopic slides and photomicrographs will be presented showing the use of the Dioxan-paraffin technic in making sections of various amphibian eggs and embryos. Yolk laden cells are not appreciably hardened by Dioxan and cracking of the sections is reduced to a minimum by this technic.

D45. Orthoscopic drawings, photographs and casts of material illustrating the topography of the ductus arteriosus in the fetus, newborn and young infant. Irving Rehman, Departments of Anatomy, College of Dentistry and the Graduate School, New York University. (Introduced by Gustave J. Noback.) (See abstract no. 91.)

D46. Correlation between ocular stimulation and spermatogenesis in the English sparrow, Passer domesticus Linnaeus. A. R. Ringoen and A. Kirschbaum, Departments of Zoölogy and Anatomy, University of Minnesota. (See abstract no. 93.)

D47. Effects of x-radiation on karyokinesis in Tettigidae and Acrididae. W. R. B. Robertson, Department of Anatomy, State University of Iowa.

Young adult males of approximately the same hatching age were treated with 3000 roentgens at the average rate of 87 units per minute; Coolidge tube, target distance 30 cm.; voltage 130 kv.; current 5 ma.; 1 mm. Al filter. Other lots under similar conditions received, respectively, 6000, 8000, 10,000 roentgens. Individuals from each lot were fixed at intervals after raying as follows: immediately, 45 minutes, 90 minutes, 3 hours, 6, 12, etc., up to 12 days. Controls from the same lots were fixed at the same times.

Chromosomes at late prophase, at metaphase, and early anaphase were most affected, those at telophase next. Agglutination of sister chromatids, or of the chromosomes as a group or in groups, prevented or retarded cell division but the progress of the spindle and non-chromatic mechanism seemed relatively little affected, except indirectly through the effect on chromosomes.

Among many, the following conditions resulted: unequal-sized daughter or sister cells with correspondingly small or large nuclei; non-nucleated cell fragments; giant tetraploid cells; giant cells with multi-polar spindles, or, in later stages, multi-nucleated; giant uni-nuclear spermatids with two, four or eight sets of some of the cytoplasmic structures; pycnotic nuclei with attendant increase in cell size but ultimate cell degeneration.

Cells which had had nuclei in the 'resting' stage at irradiation seemed quite normal.

D48. Fiber and cellular degeneration following temporal lobectomy in the monkey.

R. Wayne Rundles, Department of Anatomy, Cornell University Medical College, Ithaca. (Introduced by James W. Papez.)

Bilateral removal of the temporal lobes of two monkeys, a mangabey monkey and a baboon, was accomplished by Doctor Dusser de Barenne. For the anatomical investigation both brains were cut serially and alternating sections stained by the Pal-Weigert and cresyl violet methods. The main conclusions drawn from this work are: 1) Commissural fibers traversing the splenium of the corpus callosum and the anterior commissure have degenerated. 2) There is evidence of degenerated cingulo-temporal fibers and association fibers passing via the extreme and external capsules. 3) Optic radiations from the lateral horn and macular portion of the lateral geniculate body are interrupted. Retrograde degeneration is found in the geniculate nucleus and normal striate cortex can be distinguished from that which is deafferented. The stripe of Gennari-Vicq d'Azyr persists in deafferented striate cortex and is not, therefore, composed of terminal ramifications of geniculo-calcarine fibers. Deafferented striate cortex is characterized by a shrunken and densely stained layer IVc. 4) Both magnocellular and parvocellular portions of the medial geniculate nucleus have cortical connections. The suprageniculate nucleus does not. 5) Evidence regarding the cortical connections of the pulvinar is provided. 6) There is no evidence of temporo-pontile fibers. 7) Retrograde degeneration in the ventral nucleus of the thalamus, pars arcuata, resulted from subcentral as well as lower postcentral lesions.

D49. A graphical method of stereoscopic reconstruction. F. C. Sauer, late assistant professor of zoölogy, University of Wichita. (Prepared by Mary Elmore Sauer, Department of Anatomy, University of Alabama; introduced by Franklin S. DuBois.)

A modification of usual graphical procedures is employed to produce a perspective picture of an object from measurements in three dimensions of points in the object. A stereoscopic mate for this drawing may be readily constructed by the use of a simply ruled transparent sheet which gives directly the new location of each point. The method is best adapted to the representation of structures that can be shown as points and lines, and is applicable to reconstructions of sectioned material as well as to actual or hypothetical gross objects.

D50. The involution of the great or frontal fontanella. Richard E. Scammon, the Graduate School and Institute of Child Welfare, University of Minnesota.

The great or anterior fontanelle has been studied, by tracings on muslin strips, from several hundred healthy infants, clinically free from rickets, in the first 2 years, and its diameter and area have been determined and related with total body length, age, and horizontal head circumference. The results of this study are in graphic form. A series of tracings of the fontanelle at the maximum, minimum, third and fourth quartiles, and median levels of area are presented in full size. Graphs of the area of the fontanelle are presented for various intervals of age, total body length, and horizontal head circumference. They include the general course of decrement and the variability of the structure. The geometry of the relation of the area of the fontanelle to its oblique diameter is presented in a series of graphs and geometrical constructions and a semi-rational formula for this relation is developed. It has been found that Elsässer's measurement, which is commonly used for estimating the size of the fontanelle, is quite inaccurate for determining the area of the space, but that a simple geometric expression can be used for this relation. No consistent sex differences were found in this study.

D51. The calcification and growth rhythm in the teeth of mammals. Isaac Schour, Department of Histology, College of Dentistry, University of Illinois. (Illustrating round table paper no. 101.)

D52. Leg transplantation in albino rats. Justin V. Schwind, Department of Anatomy, Loyola University School of Medicine. (Introduced by R. M. Strong.)

Legs were transplanted to various locations on other individuals. In some cases, a hind leg was transferred from one animal to the back of another. The transfer was made in several steps, the leg being only partially amputated from the donor and the distal cut surface being attached to a denuded surface on the back of the recipient of the leg. While a union was being made between the leg and its new host, the leg received nourishment from its original owner, which was later removed.

The new leg was innervated by grafting the lateral branch of the host animal's own right sciatic nerve to the sciatic vein of the new leg. After a time, movement could be elicited in the muscles of the new leg upon stimulation of the right hind leg from which the nerve had been taken. Motion picture records were made of the grafted leg undergoing flexion and lifting a weight suspended by the toes. Lantern slides also will be shown.

Histological preparations of the tissues at varying times after transplantation, show few apparent changes in these tissues.

D53. a) *Carbol-thionin-stained sections to show the changes in the olfactory bulbs associated with 'stunting.'*

b) *Dissections of the cerebral hemisphere from the lateral surface to the level of the lenticular nucleus in man, chimpanzee, monkey, dog, cat and rabbit.* Carl G. Smith, Departments of Biology and Physiology, University of Toronto. (Introduced by E. Horne Craigie.) (No abstract.)

D54. Erythropoiesis in the liver (in vitro) of a 5-day chick embryo. Christianna Smith, Department of Zoölogy, Mount Holyoke College.

Livers of 5-day chick embryos were cultivated for 48 hours in embryonic tissue juice and plasma in hanging drop preparations. The explants were fixed in alcohol and stained in Giemsa. In the zone of growth may be seen groups of erythroblasts with some cells in mitosis. Vascular tubes are also present containing erythrocytes and in one case a flask-like expansion of endothelium is filled with cells containing varying amounts of hemoglobin and some cells in mitosis.

D55. Fetal respiration in the rabbit and human. F. F. Snyder and Morris Rosenfeld, Department of Obstetrics and the Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University. (See abstract no. 105.)

D56. Motion pictures showing the development of the salamander (Amblystoma punctatum). L. S. Stone and T. C. Kramer, Department of Anatomy, Yale University School of Medicine.

Details of cell division, the formation and closure of the blastopore, the formation and closure of the neural folds and all the periods of development up to the feeding stage are shown. Scenes showing the typical habitat in which the animal lays its eggs, the first feeding reactions and circulation in the larval tail are found in appropriate sections of the film. The story closes with a view comparing the adult with younger stages in development.

D57. A study of the developing limb following operative changes in orientation of the embryonic rudiment with respect to nearby areas. F. H. Swett, Department of Anatomy, Duke University School of Medicine. (No abstract.)

D58. The vascularity of the frog hypophysis. S. J. Taylor, Department of Biology, University of Toronto. (Introduced by E. Horne Craigie.)

The relative vascularity of three parts is determined quantitatively. Preliminary results with six frogs indicate that pars anterior, pars intermedia, and pars nervosa, respectively, have a richness which, when expressed in terms of capillary surface area, is in approximately the proportions 4: 1: 6. The demonstration will consist of sections of injected and uninjected hypophyses.

D59. The reaction of mesenteric lymphatics to drugs of different hydrogen ion concentrations. Richard L. Webb, Department of Anatomy, University of Illinois. (No abstract.)

D60. Genesis of respiration in turkey and domestic fowl. William F. Windle, Northwestern University Medical School, and Sir Joseph Barcroft, Cambridge University. (See abstract no. 122.)